AN ABSTRACT OF THE THESIS OF

Sandra Neidetcher for the degree of Master of Science in Oceanography presented on July 30, 2012.

Title: Implementation of a Gross Anatomical Maturity Key for the Study of Spawning Phenology and Geography of Pacific cod (*Gadus macrocephalus*)

Abstract approved:

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Lorenzo Ciannelli

Pacific cod (*Gadus macrocephalus*) is an important species, both economically and ecologically in the Bering Sea and Aleutian Islands (BSAI). However, little is known about its spawning dynamics. To address this knowledge gap, I developed a gross anatomical maturity key for Pacific cod to assess temporal and spatial patterns of reproductive maturity. Gross anatomical maturity keys estimate reproductive maturity by categorizing changes that occur in appearance of ovaries during maturation. Because maturity keys are based on characteristics that change on a continuum, stage assignment can be subjective particularly for ovaries in transition (displaying characteristics of more than one stage). Histological processing is often used to verify maturity key staging by estimating the maturity of individual oocytes from within assessed ovaries. I compared individual oocyte development through histological processing with gross anatomical stage assignments to evaluate the accuracy of Pacific cod maturity key using two approaches. First, assumptions made in delineating advancing characteristics between stages during the development of the key were analyzed by comparing oocyte development from ovaries assigned to key maturity stages by the researchers who designed the key. Secondly, the consistency in the use of the maturity key was addressed by comparing stage assignments to oocyte development for multiple data collectors employing the maturity key in the field. Misclassification rates for the key designers were 22% and field samplers using the key resulted in misclassification rates of 43%. While the misclassification rates are

high, the mismatch between histological assessments and gross maturity was very narrow and most often assigned correctly to an adjacent stage. Misclassifications occur most often in the vitellogenin stages where criteria for delineating between stages both histologically and through the use maturity key are based on relative changes in size and color of the ovaries.

By using morphological changes to track ovarian maturation, gross anatomical maturity keys provide an easily applied and inexpensive method for the collection of large quantities of data. Maturity data collected by observers aboard commercial fishing vessels in the BSAI were used to construct maps showing spawning sites along the continental shelves of the BSAI between the 100 and 200m isobaths. Spawning stage data for days where a high percentage of spawning was observed ("hot days") were mapped to show areas of high intensity spawning ("hot spots"). Hot spots were identified north of Unimak Island, in the vicinity of the Pribilof Islands, at the shelf break near Zhemchug Canyon, and adjacent to the central and western Aleutian Islands. Further analysis of spawning and spent stage data suggests spawning phenology was consistent during the three study years with varying climate conditions, though variation was seen in the duration of the spawning season among years and regions within years.

While the processes determining the timing and location of spawning are poorly understood, knowledge of the above patterns allows further examination of how Pacific cod spawning correspond with environmental conditions throughout the season and between years of varying climate patterns. This work provides an initial description of the distribution and phenology of Pacific cod spawning along with a generalized description of oceanographic features observed at hot spot locations during the spawning season.

Implementation of a Gross Anatomical Maturity Key for the Study of Spawning Phenology and Geography of Pacific cod (*Gadus macrocephalus*)

by
Sandra Neidetcher

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Sandra Neidetcher, Author

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Implementation of a Gross Anatomical Maturity Key for the Study of Spawning Phenology and Geography of Pacific cod (*Gadus macrocephalus*)

Main Introduction

Effective fishery management, particularly for the expanding integrated approaches of ecosystem and multiple species assessments of the 21st century require extensive knowledge of fish life history strategies. Life history traits including size at maturity, fecundity, larval growth rates, duration of spawning season, the number of spawning events, and the level of parental investment develop as trade-offs in reproductive strategies. These traits, linked to a species life history strategy, are a result of evolutionary success and are likely driven by variations in environmental conditions (Winemiller 2005). Features of the oceanic landscape such as currents and hydrography interact with bathymetry and topography to create a complex environment where variations occur from small to decadal scale and within which marine species must adapt. Knowledge of life history parameters allows researchers a better understanding of interactions between marine species and the ocean environment, and allows for the examination of variations in shifts in environmental constraints over space and time. For this thesis, I describe the implementation and the analysis of a tool developed to assess patterns in the geography and phenology of Pacific cod spawning Bering Sea and Aleutian Islands (BSAI).

Pacific cod (*Gadus macrocephalus*) range from the Sea of Japan across the North Pacific Rim to the California coast (Hart 1973) and is an important groundfish species in Alaska, both ecologically and economically. Pacific cod are predators of other demersal fishes (primarily walleye pollock) and crustaceans such as crab (Livingston and deReynier 1996) and they are prey for top predators, including endangered Steller sea lions (Sinclair and Zeppelin 2002). With the average catch from 2001 to 2005 of around 200 metric tons and valued at approximately 100 million dollars, the commercial Pacific cod fishery in the BSAI is second only to that of walleye pollock. Though catch rates have fluctuated from 2006 to 2009, a higher market price particularly in 2006 caused the ex-vessel value

doubled to 205 million tons. The price per lb in 2009 was more consistent with the early 2000s and ex-vessel value dropped to 88 million dollars in 2009 (Hiatt et al. 2010).

Pacific cod is thought to have originated from an Atlantic gadoid that dispersed to the Pacific Ocean with the opening of the Bering Strait during the mid-Pliocene 3.0 to 3.5 million years ago (Grant 1987). Life history strategies between Atlantic cod (*Gadus morhua*) and Pacific cod are similar though deviate in reproductive strategies. Both species are categorized as intermediate strategists (King and McFarlane 2003) on a triangular scaling model comparing general patterns of variation in life history patterns (Winemiller and Rose 1992). Intermediate strategists, sharing characteristic between Winemiller's opportunistic and periodic strategies are highly fecund, provide little parental investment post-spawning, and experience life spans between 10 to 20 years. Ormseth (2007) assessed the low level of maternal contribution in Pacific cod and suggested that larval survival was under environmental control. Further Ormseth suggested Pacific cod life history strategy may include longevity to match environmental variation while high fecundity rates comprise a strategy that ensures high survivorship during favorable environmental conditions.

In the BSAI Pacific cod inhabit the continental slopes and shelf, most often at depths to 300m (Matarese et al. 2003). Adult Pacific cod are dispersed across the continental shelf of the eastern Bering Sea during the summers, but then migrate in late winter, showing a high level of site fidelity, to form dense spawning aggregations along the continental slope and shelf near Unimak and the Pribilof Islands (Shimada and Kimura 1994). Spawning typically occurs at depths of 40 to 265 m (Palsson 1990), though there has also been spawning reported in shallow coastal areas (Karp 1982). Pacific cod have a relatively short spawning season which occurs in the late winter and early spring (Foucher and Westrheim 1990). Studies have shown that the spawning season occurs slightly later at higher latitudes (Westrheim 1996). Ovarian development in this species

is shown to be synchronous and eggs are released in a single batch. Pacific cod are highly fecund, releasing approximately 1,000,000 - 1mm diameter eggs per individual annually.

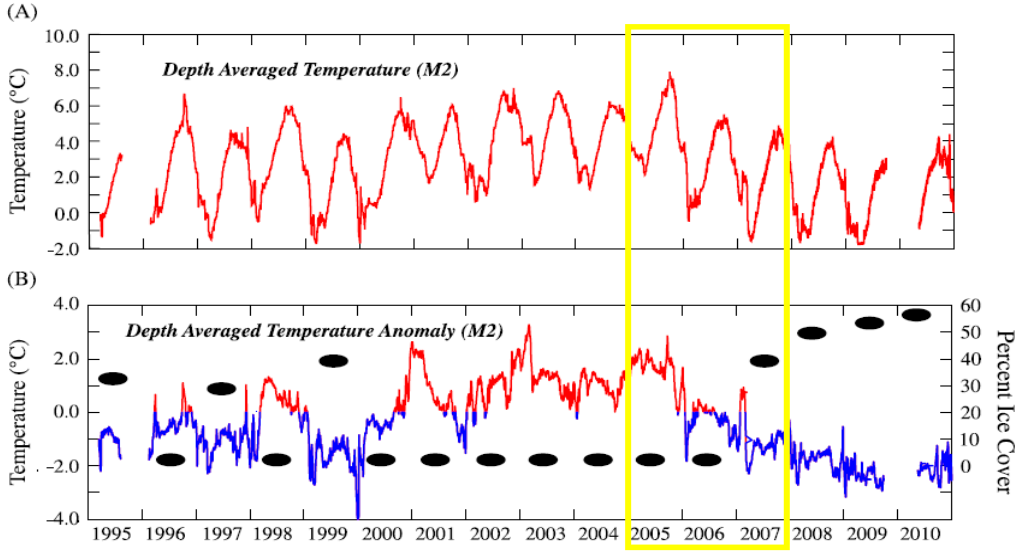
Knowledge of spawning biology provides the basis for understanding patterns in species dispersal, migration, and population connectivity. Life history parameters, i.e., the size or age of maturity, and fecundity may vary for populations and may be subject to changing environmental conditions. Tracking spawning attributes, such as timing and location, may provide valuable insight into the condition of fish population and may provide a starting point to evaluate changes in stock abundance and distribution.

Researchers have developed various methods to identify and monitor spawning populations. For example, tagging projects have provided much of our current knowledge of Pacific cod migrations and the sites of spawning aggregations (Shimada and Kimura 1994, Shi et al. 2007). However, to identify geographic and phenological distributions of spawning, release of tagged fish must occur prior to the spawning season and recovery of tagged fish must occur on the spawning grounds. Tagging efforts may be too cost prohibitive and limited in scope to adequately identify spawning locations for such a wide ranging species as Pacific cod. Additional methods used to determine spawning locations include ichthyoplankton research cruises which employ small mesh nets and target surface waters as well as discrete depths in the water column to collect spawned eggs and larval stages. However, because Pacific cod eggs are demersal (Matarese et al. 2003, Laurel et al. 2007), they are rarely captured in ichthyoplankton research nets. Though valuable information is provided through these efforts primarily for later stage larvae, surveys are often limited in temporal and spatial coverage and are not conducted throughout the spawning range for Pacific cod. Because of these sampling limitations and lack of life history research effort focused on this species (Abrookire et al. 2007), little is known about the spawning patterns of adult, or the distribution and

movement patterns of early life stages of Pacific cod (Shimada and Kimura 1994, Westrheim 1996).

Anatomical maturity keys characterize morphological changes that occur during ovary development to estimate reproductive maturity at the gross visual scale while histological processing allows the examination of individual oocyte development at the cellular scale. These methods have been used to study the onset and duration of the spawning season by estimating the reproductive condition of adult fish (Parent and Grier 2004). Gross anatomical keys allow for an inexpensive and easily deployed tool for estimating maturity stages in the field; however, keys are often considered subjective especially for ovaries in transitional stages of development (West 1990). Histological staging requires time consuming and expensive laboratory processing, though this method is often recommended to validate anatomical keys (Hunter et al. 1992).

Figure 0.1 Daily depth-averaged temperature and temperature anomalies for the eastern Bering Sea from 2005-2007. The ovals in the lower panel indicate the percent of ice coverage in March and April (Stabeno et al. 2012).



The objectives of this research include: 1) the development and implementation of a gross anatomical maturity key for Pacific cod, 2) evaluating the accuracy of the key using histological processing to estimate individual oocytes maturity from anatomically assessed ovaries, and 3) examining temporal and spatial spawning patterns of Pacific cod in the BSAI using maturity data collected by observers aboard commercial fishing vessels. Collectively these objectives will provide information for the timing and location of Pacific cod spawning from data gathered over a broad spatial and temporal scale, expanding our knowledge of spawning patterns including the spatial and temporal connectivity of spawning locations.

In chapter 1, I describe the development and implementation of the gross anatomical maturity key. This chapter also describes the use of histological processing to evaluate the ability of the key to consistently stage ovary maturation. Specimens collected by AFSC research biologists were used to assess the accuracy of the key stage descriptions, while observer specimens were used to assess the accuracy in the interpretation of key descriptions.

In chapter 2, observer assessed maturity data were used to examine spawning patterns. Alaska Fishery Science Center (AFSC) observers, assigned to commercial vessels in the BSAI were supplied with the Pacific cod gross anatomical maturity key and asked to assess maturity along with standard biological data collected on individual fish. Observer-assessed maturity data from 2005 through 2007 provide an opportunity to evaluate spawning patterns from three very different climate conditions. Water temperatures were anomalously high in 2005, while 2006 was a year of transition to a colder regime (Rodionov et al. 2005). The ice extent for 2007 was one of the highest on record reaching the Outer Domain, the Pribilof Islands, and the Alaska Peninsula east of Unimak Island (Fig. 0.1).

As the knowledge of the timing and location of spawning over broad spatial and temporal scales were made possible through this work, additional efforts were made to describe spawning and the generalized oceanography of the spawning locations. The large spatial scale of observation allowed the comparison of spawning patterns across regions, including a comparison of small scale variations associated with individual spawning sites over three years of varying climate conditions.

CHAPTER 1: MATURITY KEY DEVELOPMENT AND EVALUATION

Introduction

Pacific cod support an important commercial fishery in the Bering Sea and Aleutian Islands (BSAI) with commercial fishers targeting winter spawning aggregations. Still, little is known about the spawning patterns or the distribution and dispersal patterns of early life stages for this species (Shimada and Kimura 1994, Westrheim 1996). Knowledge of reproductive biology is required for effective fishery management and information on the gonadal maturation of individual fish is an important component of this knowledge (West 1990). Calculations of stock abundance include estimates of stock productivity where maturity estimates are used to assess age or size at reproductive maturity. Fishery managers are interested in the geography and phenology of spawning to better understand patterns in population distributions and fishery exploitation. Additionally, identifying spawning patterns, and variations in those patterns, provides information on stock structure and the impacts of changes in a species' environment. Pacific cod are currently managed as two separate stocks; the Bering Sea stock and the Aleutian Island stock (Thompson and Dorn 2003).

Multiple methods are available for assessing reproductive maturity from fine-scale estimation of the maturity stage of individual oocytes through histological processing to a more generalized assessment of gross anatomical changes seen in the whole ovary during

maturation. Histological processing allows researchers to identify structural changes at the cellular level, and as such, this method is considered to be the most accurate (Hunter and Macewicz 2003). Because histological assessment of tissue specimens requires expensive laboratory processing and time consuming microscopic analysis, practical sample sizes are often limited. Gross anatomical keys require no additional processing and are easily applied in the field, thus allowing for more extensive data collections. However, maturity keys are often considered subjective, particularly for gonads in transitional stages of development (West 1990). For example, key descriptions such as those regarding color and relative size may be interpreted differently among users. To evaluate the accuracy of maturity keys, many studies compare multiple methods for assessing maturity (Hunter and Macewicz 2003).

During a series of tagging research cruises in the Bering Sea, members of the AFSC Fisheries Interactions Team (FIT) began assessing Pacific cod reproductive development to better understand movement patterns. As a member of FIT during cruises in 2002, I assessed ovary development using maturity keys available in the literature (Hirshchberger and Smith 1983, Tyler 1995). After noting the variable interpretation of key descriptions by FIT members, I worked to clarify key stages, expanded the key descriptions, and include digital photos of key stages. Additionally, I collected ovary tissue samples for histological analysis to determine if key assignments corresponded to the maturation level of individual oocytes present in the assessed fish.

Histological methodologies were used to compare the development of ovarian structures at the microscopic scale with gross anatomical stages assignments made by Fit cruise members or observers on the same fish in the field. Determining how microscopic structures align with gross anatomical staging through the spawning season allowed the evaluation of key development.

Fisheries observers onboard vessels in the BSAI were asked to assess ovarian maturity using the maturity key developed during the FIT cruises. To assess observer application of the maturity key, a number of observers were asked to collect ovary tissue specimens for histological evaluation. A comparison of these specimens to the maturity key assignments allowed for an evaluation of variability in the interpretation of the maturity key descriptions*;* to compare the use of the maturity key between key developers (considered Pacific cod maturity experts) and fishery (field samplers with minimal training in maturity estimation).

Methods

Key development

Initial maturity stage assessments during FIT cruises were estimated using Pacific cod maturity keys from previous studies (Hirshchberger and Smith 1983, Westrheim 1996). With a number of biologists assessing gonad maturity, attempts were made to reduce inconsistent assessments by focusing on specific visual characteristics that change as the spawning season progresses. Three gross anatomical characteristics were addressed; 1) the overall size of the gonad in relation to the size of the abdominal cavity; 2) the color and translucence of the ovary; and 3) the adhesiveness of the oocytes, specifically, the degree of binding in the follicle lamella. In addition to the above characteristics, the presence or absence of vascularization and the visibility of oocytes to the unaided eye through the ovary wall were included in the key description. Conditions such as flow of eggs from the ovary with applied pressure helped define the spawning stage. Descriptions from the literature were expanded and photographs were included to clarify transitional changes during maturation. The resulting Pacific cod gross anatomical maturity key was based on six maturity stages for: 1) Immature, 2) Developing, 3) Prespawning, 4) Spawning, 5) Spent and 6) Resting (Figure 1.1). Though the key provides maturity stages for male testis, the data presented for this thesis will focus on females only.

Figure 1.1 FIT six-stage Pacific cod gross anatomical maturity key developed by S. Neidetcher.

Pacific Cod Maturity Codes (female)

Stage Description

1. Immature

Gonads small, close to vertebral column, may be difficult to see. Ovaries appear as pink or transparent paired sacs, no oocytes are visible to the eye.

* Look for transparent or pinkish coloration. There may be slight silver or dark blotching on the surface, but the ovary should be small and new looking as shown.

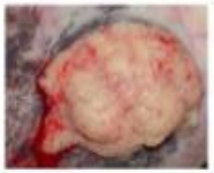
2. Developing

Gonads small, to about ½ the length of body cavity. Ovaries form 2 tapered, distinct lobes having well-developed blood vessels. Transparent and/or opaque orange oocytes are distinct and visible through the ovary wall. Oocytes stick together forming a solid mass.

* The ovary shown is fairly small, as stated above the developing ovary can be up to half of the body cavity

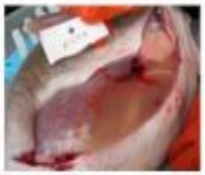
3. Pre-spawning

Ovaries form 2 large distinct lobes. Most eggs appear as mature clear ova, some oocytes remain interspersed throughout the ovary. Ova are less adhesive resembling the consistency of Cream of Wheat (the breakfast cereal).



4. Spawning

Eggs run under slight pressure to the body. Ova are loose in the ovary. (The ovary was cut open sexing the fish shown at right. The eggs (ova) fill the abdominal cavity and flow freely into the bin.) * To differentiate ovary stages 2 through 4 look at the adhesive quality of the eggs. Stage 2 eggs form a solid mass while stage 3 eggs are looser and stage 4 eggs flow freely.



5. Spent

Gonads are still large, but appear flaccid and watery. Ovaries may contain remnants of disintegrating ova and associated structures.

6. Resting

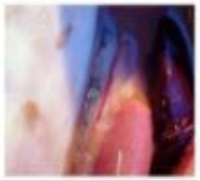
Ovaries small, firm, may have some black or silver color. No oocytes are visible to the eye.

Pacific Cod Maturity Codes (males)

Stage Description

1. Immature

Gonads small, close to vertebral column, may be difficult to see. Testes translucent "bumps" on thread-like strands closely associated with, and often indistinguishable from, the gut mesentery.



2. Developing

Gonads small, to about ½ the length of body cavity. Testes appear uniformly ribbon-like, swollen in size, and take on an opaque or increasingly whitish color.

3. Pre-spawning

Testes are large, swollen white and highly convoluted "leaf-lobed" ribbons filling the entire body cavity. Milt is emitted when wall is compromised (pinched).

4. Spawning

Testes milk freely under slight pressure to the body.

5. Spent

Gonads are still large, but appear flaccid and watery. Testes appear bloodshot. There may be some areas of the testes that still contain milt.

6. Resting

Key developed by Sandi Neidetcher, Alaska Fisheries Science Center,
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Gross anatomical maturity key for Pacific cod

The six maturity stages described in this key were determined by observing morphological changes that occurred as the spawning season progressed during an early research cruise in 2002. Shifts in the appearance between gonads characteristics allowed for the delineation of six identifiable stages based on size, color, and the consistency of oocytes in the ovary. These changes in characteristics over the spawning season are presumed to align with changes that occur within the ovary during maturation.

Immature ovaries are small in comparison to more mature stages; they are tubular in shape and lie against the dorsal wall of the abdominal cavity. At this stage, ovaries may be difficult to distinguish from testes for very small fish. Oocytes are not distinguishable through the ovary wall and the color is pinkish to translucent.

With developing and prespawning stages, oocytes can be clearly seen through the ovary wall. These stages include the accretion of vitellin, or yolk, in the individual oocytes. Ovaries in the developing stage increase in size as oocytes expand with yolk development Smaller developing stage ovaries may not be much larger than an immature stage ovary, though the coloration will range from orange to pale yellow. The developing stage ovaries are typically highly vascularized, with veins easily visible beneath the ovary surface. The size division between developing stage and the more advanced prespawning stage identified described in ovary size in relation to the abdominal cavity. The developing stage is identified when the ovary occupies *less* than half the volume of the abdominal cavity, while the prespawning stage is identified when the ovary occupies *more* than half of the abdominal cavity.

The oocytes within the developing stage ovary are tightly bound in the follicle by microvilli providing nutrients to the oocytes. At this stage, oocytes appear to be firmly adhered to each other such that separating oocytes with a field knife is difficult. At the prespawning stage microvilli detach as the oocytes' envelope expands with increased

fluid absorption. The oocytes appear less rigid and are easily separated from each other. At the prespawning stage, oocytes are close to ovulation: the release of oocytes as mature ova into the lumen.

Ova flowing freely with slight pressure to the abdominal cavity are the singular qualification for determining spawning stage fish. The ovary collapses upon release of ova during spawning. As a result, when most of the ova are released during spawning and during the spent stage, ovaries appear flaccid and dark reddish in color. Fluid, with blood and residual hydrated oocytes may be observed when spent stage ovaries are sliced open. As the ovary tissues contract during recovery, the external color remains dark and grayish. During the resting stage between annual spawning seasons, ovaries appears small in comparison to the developing stages, are dense in structure and have a rounded triangular shape.

The maturity key was developed by observing the maturation process during the spawning season. Few samples were collected through the summer and fall and therefore key descriptions do not specifically address the gross anatomical characteristics of ovaries outside the spawning season, although the resting stage is assumed to apply to these periods.

Additionally, although the maturity key includes characteristics of male as well as female gonads, we focused our assessment of the use of the key and data analyses on females only. Male marine fish often mature to the spawning stage earlier than females and remain at the spawning stage for a longer time than females, such that female reproductive state appears to drive the temporal and spatial distribution of spawning. Although initial evaluation of the temporal and spatial patterns of spawning appear to be similar for male and female Pacific cod, few resources are available for histological staging in male fish. Future efforts will include assessing the accuracy of male maturity assignments for this species.

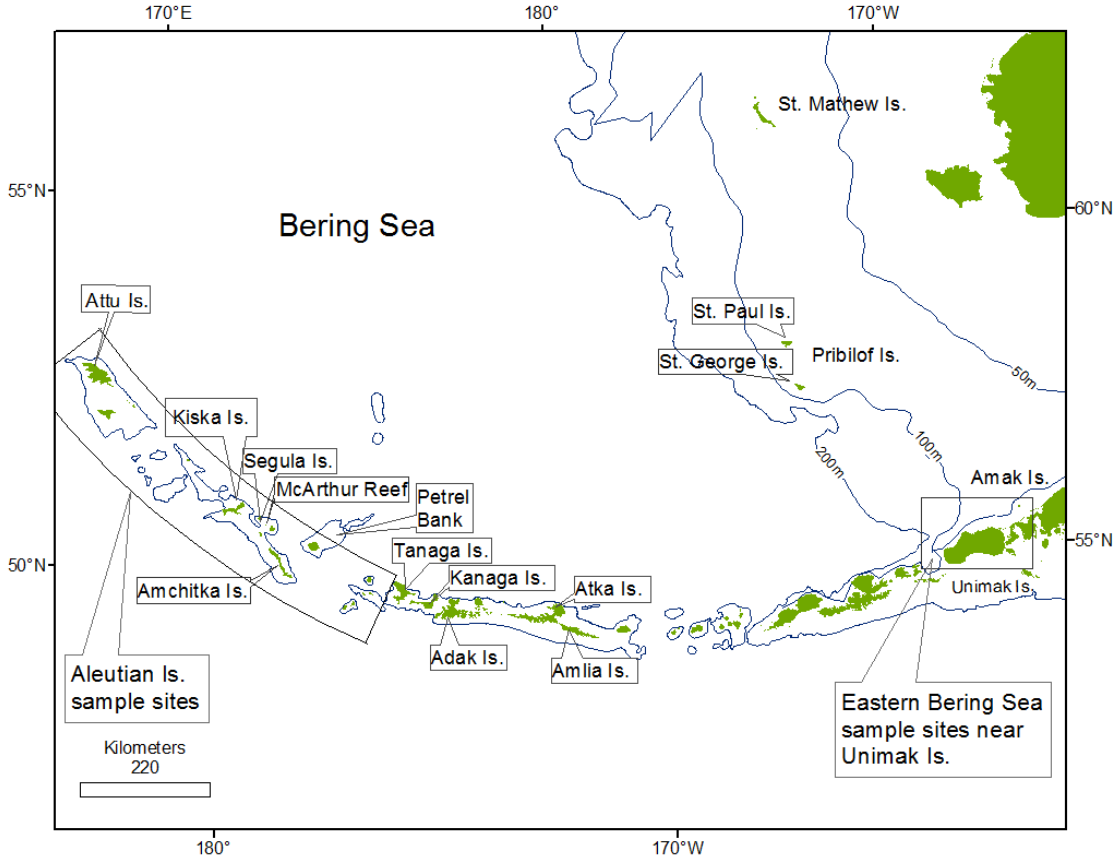
Data collection

The maturity key was deployed with FIT scientists during research cruises and with observers aboard commercial fishing vessels in the BSAI. FIT scientists and observers assigned maturity stages and collected ovary tissue from a selection of maturity-assessed fish. Ovary tissue specimens were analyzed using histological methods; this includes fine-sectioning, staining, and mounting tissue sections onto a microscope slide. Histological specimens were then used to compare the development of individual oocytes at the microscopic level with gross anatomical assignments made of the whole ovary at the macroscopic level.

FIT cruise gross anatomical maturity and ovary specimen collections

Maturity assignments were made during FIT cruises using pot gear to capture fish near Unimak Pass in the Bering Sea between 2003 and 2004. Additional maturity sampling was accomplished by a FIT researcher aboard a commercial trawler fishing various areas along the Aleutian chain in the spring of 2005 (Figure 1.2). FIT maturity sampling during 2003 to 2005 was conducted from early January to mid-April. Maturity was assessed on fish from randomly selected pots. FIT sampling included maturity stage assessments for at least 100 fish per selected pot if catches were large enough. To avoid selection bias, all fish were assessed if catch counts were below 100 fish. When catches were over 100 fish, individual fish were selected by randomly selecting baskets of fish, from rows of baskets filled when fish were moved from the sorting table to the weighing scale. Fish were assigned gross anatomical stages after slicing each fish along the abdomen and examining the ovaries to determine the sex and the maturity compared to the characteristics described in the maturity key.

Figure 1.2 Sampling areas during FIT research cruises in the eastern Bering Sea and locations in the Aleutian Islands where a FIT scientist sampled while aboard a commercial fishing vessel.



During 2004 and 2005, FIT researchers collected ovary tissue samples from a subset of maturity-assessed fish. Specimens were collected from randomly selected pots. A length-stratified method was used to ensure that collections included specimens from all length categories. Ovary specimens were removed in the field and preserved in 10% formalin for histological analysis. Additionally, the collection of digital photographs allowed the side-by-side comparison histological stages to the gross anatomical maturity stages for 50 individual fish.

AFSC observer gross anatomical maturity and ovary specimen collections

AFSC observers assessed reproductive maturation of Pacific cod in commercial catches in the BSAI starting in 2004. Observers sampled catches from Pacific cod directed fisheries, and from Pacific cod caught incidentally during directed fisheries for other species. The capture gear included trawl nets, longlines, and pots. Prior to deployment, observers were trained in use of the maturity key.

To sample commercial catches (hauls), observers used a random sampling protocol to select a subset of hauls. From these hauls, fish were selected for gender analysis and measured for length. Most observers assessed the maturity of all fish used for biological sampling. Other observers selected fish from hauls that were *not* assessed for biological characteristics when Pacific cod were available. While these hauls were opportunistically sampled, the selection of fish from the hauls was obtained using random selection techniques. Additional details concerning observer sampling protocols are available in the AFSC North Pacific Groundfish Observer Manual (2005-2007). To assess gonad maturity, the abdominal cavity was opened and characteristics of the surface of the ovaries and condition of individual oocytes were compared to descriptions and digital photos provided in the maturity key.

Observers recorded the date of the fishing event and the fishing location's latitude and longitude measured to degrees and minutes. For vessels using trawl gear, capture locations include the point at which the gear was retrieved from fishing depth. For fixed-gear vessels, the recovery location was typically the end of the gear string. Strings of pots and longlines of individual fishing hooks were often several km in length.

To assess the variability in the application of the maturity key, in 2007 and 2008 observers were asked to collect ovary tissue samples from a portion of the fish they assessed for maturity. For these collections, observers sampled fish from those used for

biological collections to fill a length-stratified quota, thus ensuring that all size ranges were represented in samples. Tissue samples were collected either by placing whole ovaries in mesh bags, or by taking a small section of the ovary and securing it in a mesh-lined tissue cassette. Gonad material was fixed in the field with 10% formalin and transported to the laboratory at the end of the fishing season.

Histological processing

Whole ovary collections were removed from the formalin solution and allowed to soak in water for up to 24 hours in the laboratory. Thin sections of each ovary were placed in a tissue cassette lined with a biopsy bag or sponge to prevent specimen contamination. Previous observation in Pacific cod histology has shown consistency in oocyte stage distribution within the ovary (Stark, 2007); therefore, thin sections were collected from the ovary where convenient to include a portion of the ovary wall and to ensure adequate penetration of the fixative. Specimen cassettes were sent to a contracted histology laboratory where tissues were dehydrated through a graded ethanol series, cleared, and embedded in a block of Paraplast. Specimens were sectioned to thicknesses of 0.003 and 0.005 mm and mounted on a slide. Harris haematoxylin and eosin were used to stain tissues.

Histological staging

Bowden et al. (1990) describe the process of oocyte maturation and suggest that the appearance of structures and conditions during the maturation process provide various landmarks that can be useful in marking transitions in development. Described below are categories where these structures and conditions are combined to best represent development stages seen in ovaries. Although prior studies suggest oocyte staging based as the most advanced oocyte structures present (Hunter and Macewicz 2003), these methods may estimate the maturity of the ovary as a more advanced stages than the gross

anatomical staging would indicate. A period of transition occurs when individual oocytes develop at slightly different rates. It is not likely that a small number of advancing oocytes affect the morphology of the whole ovary, but rather a majority of oocytes achieving a level of development produce the changes described in the maturity key. For this study, histological staging assignments were made by identifying the most abundant structures present. If this information was absent, the category assigned was the midrange of the oocyte stages observed.

No Yolk (NY)

Ovaries containing germinal vessels and perinuclear stage oocytes only, and lacking any sign of further development such as vitellogenesis, hydration, or post-ovulatory follicles, are categorized as No Yolk (Figure 1.3). These fish are identified by the maturity key as pre-vitellogenin; either immature (young fish prior to sexual maturation), or resting (sexually maturity fish lacking current oocyte maturation).

Cortical Alveoli (CA) and Early Yolk (EY)

The presence of cortical alveoli marks the beginning of vitellogenesis and an increase in size and accumulation of lipids and protein. EY, or the early accumulation of yolk droplets, may be evident without the presence of cortical alveoli. When observed, the yolk vacuoles may be distributed around the perimeter of the ovary or around the nucleus. The EY stage is defined as the accumulation of yolk where the cytoplasm of the oocytes is less then filled with yolk droplets (Figure 1.4). With early cortical alveoli structures, prior to the accumulation of vitellenin, ovaries begin to thicken from the tubular shape to a more triangular shape. The color of the ovary begins to change as yolk droplets form. The maturity key differentiates immature from developing stages by color and size. Development prior to yolk accumulation is likely identified as immature.

Beginning Nuclear Migration (BNM) and Mid Yolk (MY)

Changes that occur with the yolk droplets, along with the shape and position of the nucleus, are used to further mark transitional stages of development. The MY stage is marked by an accumulation of yolk to fill the cytoplasm (Figure 1.5). At this point, a change in the nucleus to a crescent shape and a migration to the periphery of the cytoplasm may be seen. Because the appearance of nuclear migration, an asymmetrical process, is dependent on the orientation of the oocytes in the specimen processing, the state of the yolk droplets is often a more reliable indicator of the level of maturation. Beginning nuclear migration coincides with the MY stage, and where a slight change to the shape or location in the nucleus is observed. The maturity key descriptions includes oocytes development of BNM and MY as developing stage ovaries.

Mid Nuclear Migration (MNM) and Late Yolk (LY)

Mid Nuclear Migration and Late Yolk are identified by the nucleus migrating further off center and changing into a crescent shape (Figure 1.6). The oocytes further increase in size due to hydration. The yolk droplets become increasingly clustered, with yolk-free spaces opening among the droplet groups. The maturity key description includes oocyte development of MNM and LY as prespawning stage ovaries.

Late Nuclear Migration (LNM) and Yolk Coalescence (YC)

In later stage vitellogenesis, yolk droplets coalesce then disperse in the cytoplasm (Figure 1.7). Late nuclear migration, seen as a thin, crescent-shaped nucleus in close proximity to the oocyte wall, is sometimes visible in ovaries with yolk coalescence. The progression through yolk coalescence can be seen with early droplets merging to form much larger droplets, then when droplets are fully dissolved and the nucleus disappears into the cytoplasm (Figure 1.8). Ovaries in this category often contain a number of fully

hydrated oocytes, with some already released from the follicles into the lumen. The maturity key descriptions include LNM and YC oocytes as prespawning stage ovaries.

Hydration (HYD)

At full hydration, histologically, the ova appear uniform, pink-stained, and translucent (Figure 1.9). After release from the follicles during ovulation, further hydration results in further increase in oocyte volume (to approximately 1mm in diameter). When the spawning stage as described by the maturity key is observed in the field, ova are transparent and free flowing.

Post- Ovulatory Follicles (POF)

The presence and condition of post-ovulatory follicles, along with the presence of blood cells in the lumen, identify recently spawned fish (Figure 1.10). The maturity key description includes the appearance of a flaccid and bloody ovary as a spent stage ovary.

Atresia (AT)

Oocytes and ova that fail to spawn are reabsorbed in the ovary. Atresia can be identified by the degradation of the oocytes envelope, or zona radiata (Figure 1.10). Atresia may occur when oocytes are in any stage of development and may be identified as a chorion remnant that persists long after the oocyte is reabsorbed. These structures are easily discerned from the remnants of follicles after ovulation has occurred.

Histological slide analysis

Each slide was scanned for the presence or absence of oocyte structures and conditions indicating maturation. These microscopic observations included the presence or absence of yolk droplets, the thickness of the ovary wall, and the presence of cortical alveoli. Characteristics such as quantity, distribution, and integrity of yolk droplets; the position

and shape of the nucleus; hydration; presence and condition of empty follicles; the presence of blood; and the placement of the oocytes in the lumen were noted for each slide. These structures were recorded and grouped into categories classified as histological stages using modified methods from Hunter et al. (1992) and Stark and Somerton (2002) and described in Table 1.1.

Comparisons between maturity stages and histological structures

To evaluate the accuracy of the maturity key, maturity stage assignments for individual ovaries were compared to the oocyte structures identified from those specimens. The alignment of histological structures to anatomical staging was based on the presence of oocyte structures or conditions that result in the gross anatomical changes observed in ovaries as summarized in Table 1.1. Classification tables are used to show the agreement between the two staging methods. The percent of misclassifications per maturity stage identifies the extent to which oocyte stages do *not* correspond with the maturity stage assigned in the field.

Histological cross sections showing developmental stages of Pacific cod

Abbreviations used in figures and tables

NY = no yolk
CA = cortical alveoli
EY = early yolk
BMG = beginning nuclear migration
MNM = mid nuclear migration
LNM = late nuclear migration
YC = yolk coalescence; yolk droplets fuse and start to break down
HYD = yolk droplets diffuse throughout the cytoplasm and oocyte size expands in size
EPOF = early post-ovulatory follicles
BL = blood cells
POF = post-ovulatoty follicles
ATR = atresia

Figure 1.3 – 1.10 Histological images Chapter images 10X magnification

Figure 1.3 No Yolk classification showing perinuclear (PN) and germinal vessel (GV) stage oocytes.

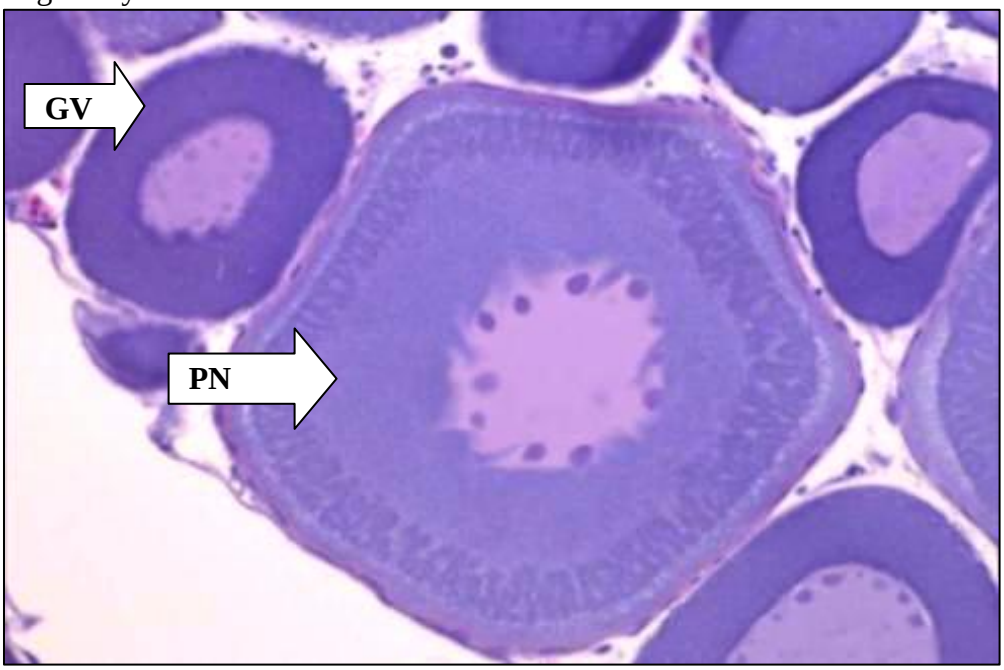


Figure 1.4 Cortical alveoli (CA) and early yolk (EY) oocytes

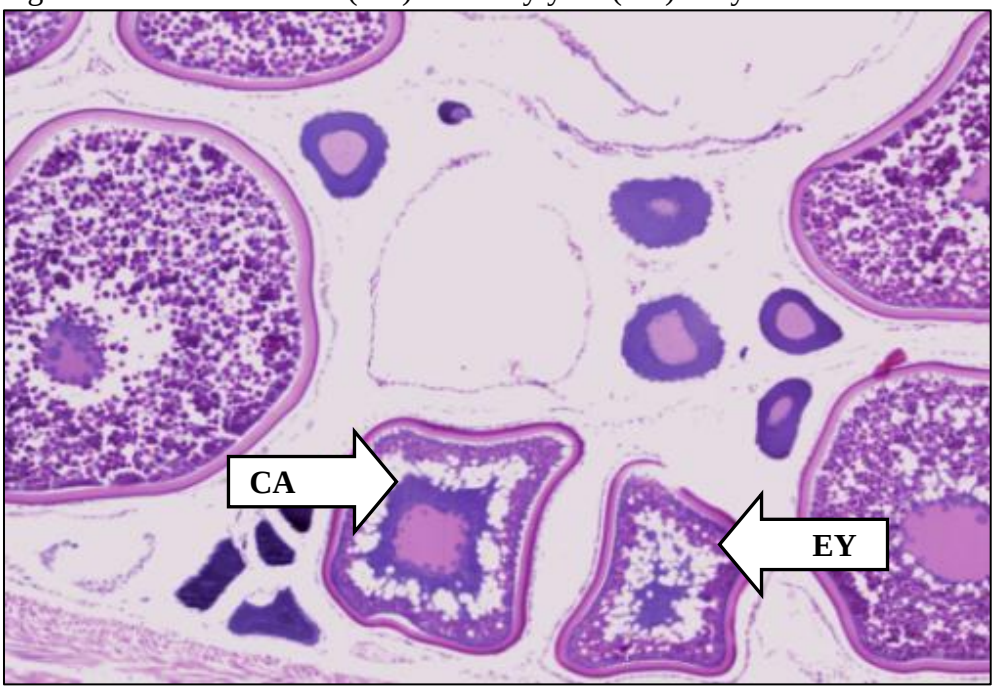


Figure 1.5 Beginning nuclear migration (BNM) and mid yolk (MY) oocytes

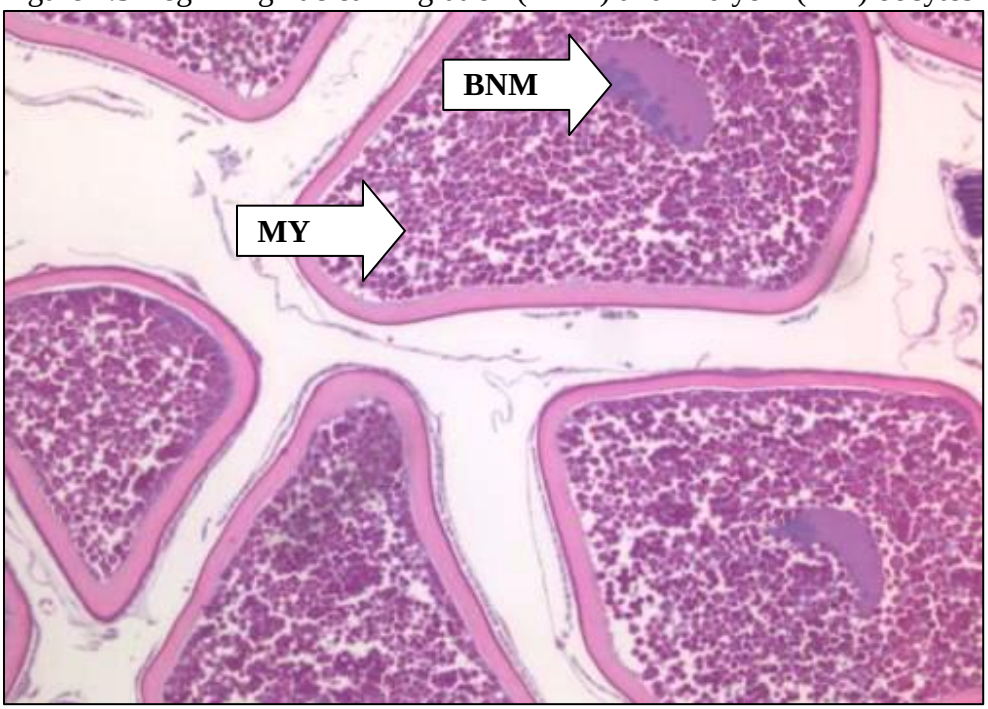


Figure 1.6 Mid nuclear migrations (MNM) in late yolk (LY) oocytes

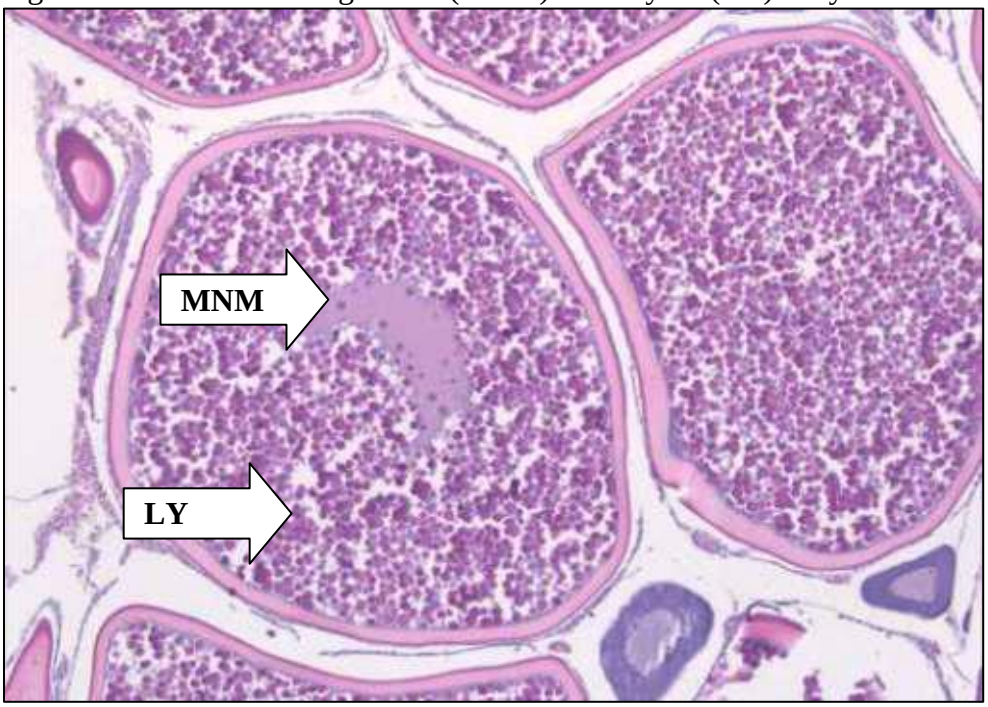


Figure 1.7 Late nuclear migration (LNM) oocytes

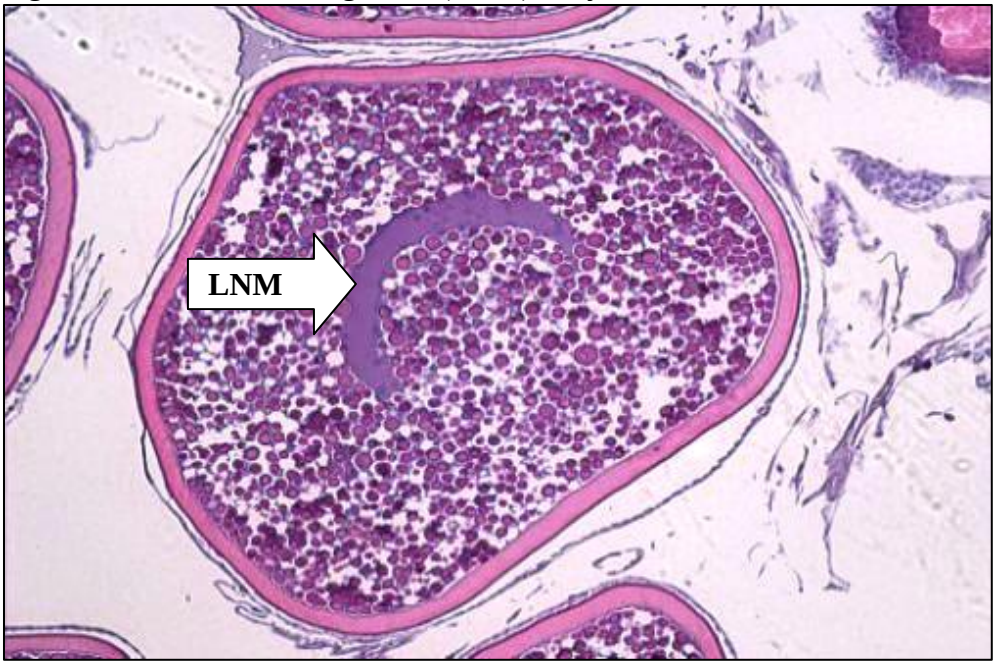


Figure 1.8 Yolk Coalescence (YC) oocytes

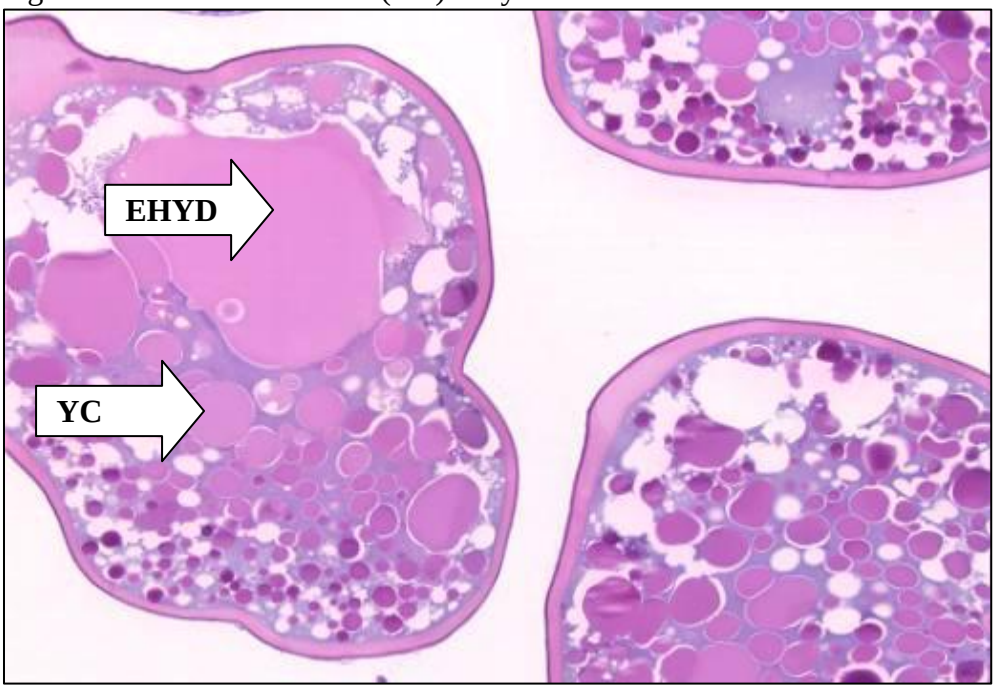


Figure 1.9 Oocyte hydration (HYD)

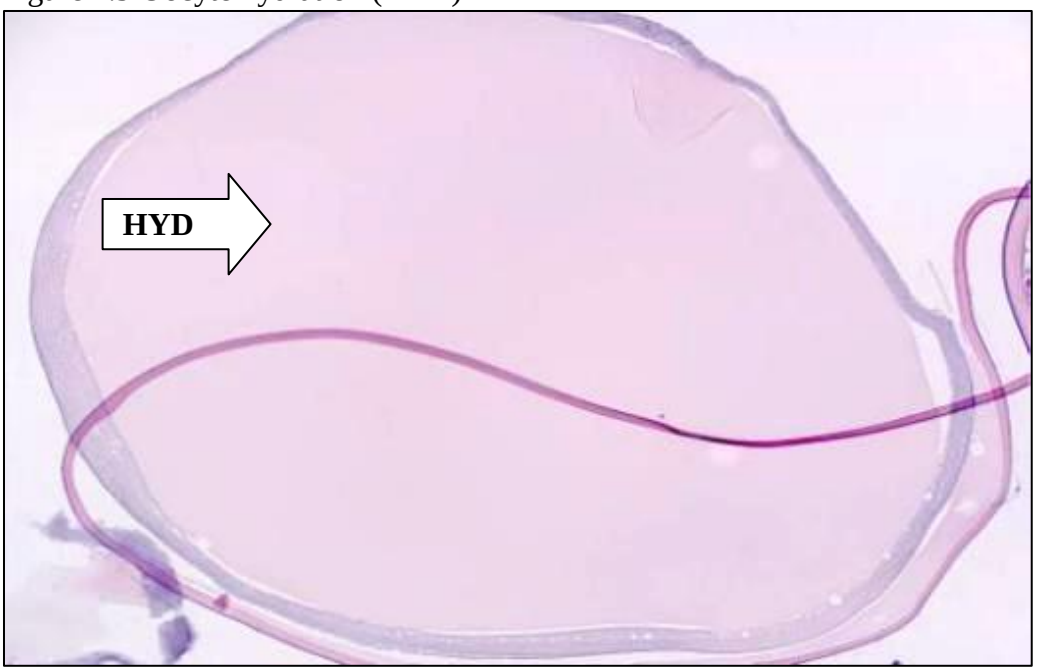


Image 1.10 Post-ovulatory follicles (POF)

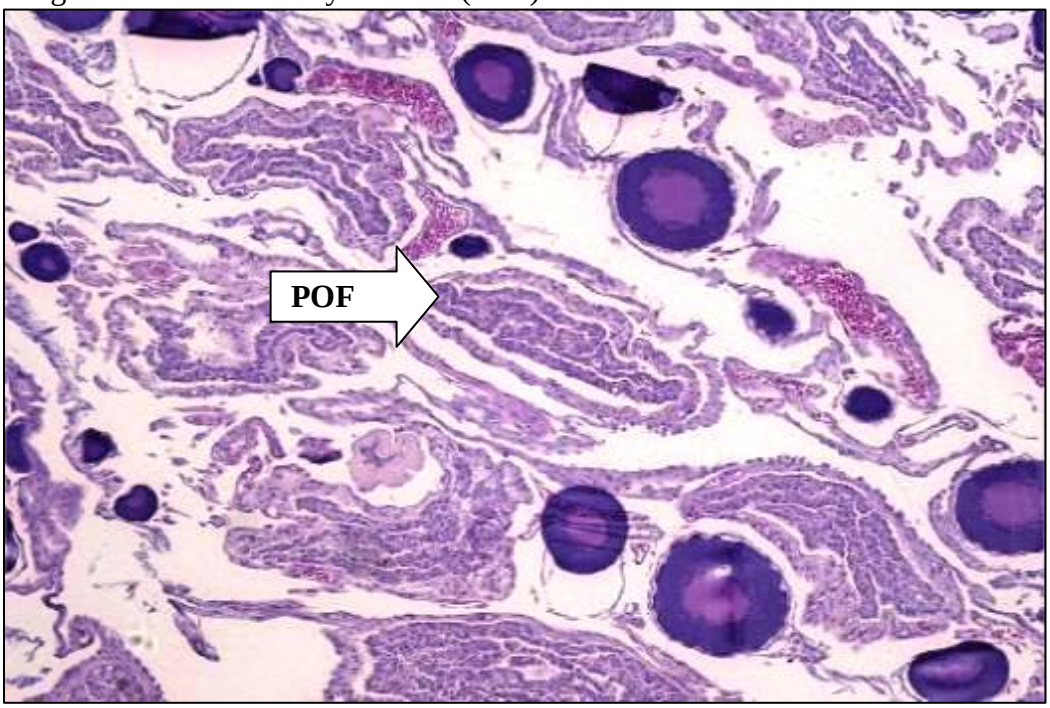


Table1.1. Description of gross anatomical maturity stages and corresponding histological oocytes classifications.

Anatomical stage	Anatomical stage description	Histological stage	Oocyte Structures	Oocyte structure descriptions
Immature	Small, pink or transparent, no oocytes visible to eye	No yolk (NY)	Germinal vesicles, perinuclear oocytes, absence of vitellogenin	Small cells with centrally positioned nucleus, nucleolus distributed along periphery of nucleus
Developing	Small to ½ the length of the abdominal cavity, well-developed blood vessels, oocytes distinct and visible through ovary wall, coloration yellow to bright orange. Oocytes difficult to separate	Early yolk (EY)	Cortical alveoli	Vacuoles around periphery of cytoplasm
			Early yolk	Few small yolk droplets to medial to vacuoles to half cytoplasm full of yolk
		Mid yolk (MY)	Beginning nuclear migration	Some oocytes with compressing and off-center nucleus
			Mid yolk	No cortical alveoli, yolk fills oocytes cytoplasm
Prespawning	Ovaries are greater than ½ the abdominal cavity. The ovary surface is translucent and mottled grayish. Oocytes are opaque and less adhesive.	Late yolk (LY)	Mid nuclear migration	Many nuclei compressed and moving to the oocytes wall
			Late yolk	Oocyte expansion allows separation between yolk droplets
		Yolk Coalescence (YC)	Late nuclear migration	Crescent shape nucleus next to oocytes wall
			Yolk coalescence	Yolk beginning to coalesce resulting in increases, variability in size.
			Early hydration	Yolk dissolving and the oocytes increases in size as more fluid is absorbed
Spawning	Ova run under light pressure to the abdomen	Hydration (HYD)	Full hydration	Hydrated oocytes in the in lumen
Spent	Gonads appear flaccid and watery. Ovaries may contain remnants of disintegrating ova and blood	Post-ovulatory follicles (POF)	Early post-ovulatory follicles	Collapsed follicles and blood cells in lumen, reabsorbing hydrated oocytes
			Post ovulatory follicles	Collapsed follicles
Resting	Ovaries are small and firm, may have black or silver coloration on surface. No oocytes are visible to eye	No yolk-thick wall (TKNY)	No yolk, thick ovary wall	Germinal vesicles and perinuclear oocytes with a thick cell wall

Results

Data Collection

Sample sizes for histological specimen collections made by FIT researchers are provided per cruise series in Table 1.2. FIT researchers consulted on gross visual maturity assessments during the 2002 cruise series, although no specimens were collected. In 2003, all researchers on board worked to clarify maturity stages and gained a consensus on transitional stages of development. For subsequent years, FIT researchers continued to consult and train new samplers. All assessments were made with at least one researcher on board who had worked to develop the FIT key. In 2005, a FIT researcher assessed maturity and collected ovary tissue samples aboard a commercial fishing vessel from locations in the central Aleutian Islands. From the FIT collections, 706 fish samples were processed and assigned a histological category.

Table 1.2 FIT histological specimen per year and gross anatomical maturity stage assignments. All collections assignments were made by FIT researchers sampling near Unimak Pass and locations in the Aleutians (*)

FIT cruise	Immature	Develop	Prespawn	Spawn	Spent	Resting	Total
2003	57	54	68	2	7		188
2004	5	69	71	6	15		166
2005	25	156	24	4	13	4	226
2005*	12	9	83	20	2		126
Total	99	288	246	32	37	4	706

AFSC observers typically work independently, although some consultation occurred with FIT researchers via email, field consults, and telephone conversation either prior to, or after collections were made. On rare occasions, a FIT researcher worked with observers at processing plants and some vessels carried two observers who had the opportunity to work together. Thirteen observers participated in collecting histological specimens from

589 fish (Table.1.3). Depending on their deployment dates, the frequency of their encounters with the different maturity stages varied.

Table 1.3 Observer histology specimen collections listed per observer by maturity stage.

Observer ID #	Immature	Developing	Prespawning	Spawning	Spent	Resting	Total
1	6	22	4				32
2	34	29	5	1		1	70
3	15	18	2				35
4	1	17	3	1		1	23
5	16	26	12	17	2	7	80
6	4	8	14	12	6		44
7	6	5	1	3	11	3	29
8	4	5	5	4	1	3	22
9	21	11	26	7	3	10	78
10		8	3				11
11	12	3	6	2	4	13	40
12	9	9	14	1	4		37
13	2	4	6	2	4	1	19
Total	156	183	119	58	39	39	593

Gross anatomical assessment and histological stage comparisons

FIT Cruises

A comparison of macroscopic maturity assignments to the oocyte structures present in the ovary specimens collected during the FIT cruises is provided in Table 1.4. The blocks running diagonally from the top left to the bottom right (shown in gray) indicate specimens where the maturity stage assignments match the histologically assessed oocyte structures as described in Table 1.1. The overall misclassification rate for FIT maturity assignments was 22%, with the highest rates occurring between the developing and prespawning stages.

The maturity key immature stage was in agreement with the corresponding NoYolk oocyte category in 93% of the ovaries identified as immature. Of the remaining 7% of the specimens that lacked vitellegenin accumulation, 2% observed to have a thicker ovary wall (TKNY) suggesting a resting stage and 3% contained POFs suggesting spent stage ovaries.

Agreement between the maturity key developing stage and histological oocyte categories Early Yolk (100) and Mid Yolk (103) was found in 70% of the specimens. Thirty percent were misclassified; 76 (26%) of the specimens contained more advanced Late Yolk oocytes, and 7 (2%) contained Yolk Coal oocytes that were misclassified as the lesser advanced developing stage. Additionally, one specimen each of NoYolk and POF structures were misclassified as developing stage ovaries.

Ovaries assigned to the prespawning maturity stage were correctly classification for 82% of the specimens, Late Yolk (186) and Yolk Coal (16). Misclassification occurred in 18% prespawning ovaries. Of these, 15% (38) were ovaries that contained the lesser developed Mid Yolk stage oocytes. Additionally, four specimens contained more advanced HYDR and POF stages.

Seventy-two percent (23/32) of the spawning stage assigned ovaries contained HYDR oocytes, while 16% contained less mature Yolk Coal (2) and Late Yolk (3) oocytes and 13% (4) ovaries contained the more advance POF structures. Misclassification occurred with the spent stages on 16% of the samples with 2 specimens contained HYDR oocytes and 3 specimens were identified histologically as NoYolk oocytes. Resting stages assignments showed the largest misclassification rates, but was also comprised of very low sample size.

Table 1.4 FIT gross anatomical assessment per histological category for the same fish. Oocyte staging is presented vertically and anatomical staging is presented horizontally. Agreement between the two measures is highlighted along the diagonal. The percent of oocyte stages misclassified as a different stage than the corresponding stage provided in Table 1.1 is provided in the stippled row.

Gross / Histology	Immature	Develop	Prespawn	Spawn	Spent	Rest	Total
No Yolk	93	1	1	0	3	0	98
Early Yolk	1	100	0	0	0	3	104
Mid Yolk	0	103	38	0	0	0	141
Late Yolk	0	76	186	3	1	0	266
Yolk Coalesce	0	7	16	2	0	0	25
HYDR	0	0	4	23	2	0	29
POF	3	1	1	4	31	0	40
TKNY	2	0	0	0	0	1	3
Total	99	288	246	32	37	4	706
% misclass	6%	30%	18%	28%	16%	75%	22%

Observer collections

Observer maturity stage assignments and histologically assessed oocyte structures from the same fish were compared as in the above section (Table 1.5). The misclassification rate for the observer collections show similar trends as the FIT data, but with a higher overall misclassification rate of 43%. As with the FIT cruise comparisons, a large portion of the misclassification occurred with the developing and prespawning stages.

Ovaries assigned to the immature stage contained primarily NoYolk oocytes with 8% misclassification as TKNY oocytes. The developing maturity stage agreement between the corresponding Early Yolk (2) and Mid Yolk (37) oocyte stages was found in 21% of the sampled ovaries. The Late Yolk (100) was the most predominant oocyte category found in 55% of ovaries identified as developing and 16% were identified as Yolk Coal (23). A misclassification rate of 9% occurred with the less advanced NoYolk (17)

oocytes category. Prespawning stage assessments were more closely aligned with a much lower (23%) misclassification rate; primary misclassification occurred with MidYolk (25) oocyte category. Spawning stage ovaries correspond to hydrated ovaries in 46% of the specimens but were found to contain less advanced vitellogenesis oocyte stages; Yolk Coal 10% (6) Late Yolk, 35% (20) and MidYolk 7% (4). This comparison for spent stage ovaries shows a similar misclassification with less advanced oocyte stages. Ovaries with POF structures were most often identified as spent, although misclassification occurred occasionally (18%) with advanced vitellogenesis and hydration oocyte stages. TKNY ovaries had the largest misclassification rate. It is likely that characteristics at the gross visual scale are not adequate to differentiate the structures in No Yolk and TKNY stages.

Table 1.5 Observer gross anatomical assessments per histological category for the same fish. Oocyte staging is presented vertically and anatomical staging is presented horizontally. Agreement between the two measures is highlighted along the diagonal. The percent of oocyte stages misclassified as a different stage than the corresponding stage provided in Table 1.1 is provided in the stippled row.

Gross / Histology	Immature	Develop	Prespawn	Spawn	Spent	Rest	Total
No Yolk	143	17	0	0	2	31	193
Early Yolk	0	2	1	0	0	0	3
Mid Yolk	0	37	25	4	0	0	66
Late Yolk	1	100	50	20	0	0	171
Yolk Coalesce	0	23	40	6	2	0	71
HYDR	0	0	1	26	3	0	30
POF	0	1	0	1	32	2	36
TKNY	11	2	0	0	0	3	16
Total	155	182	117	57	39	36	586
% misclass	8%	79%	23%	54%	18%	92%	43%

Tracking misclassification rates over time is also useful in understanding when maturity stage assignments differ from histological staging. Figure 1.4 displays correct classifications between maturity assignments and oocyte structures for data collected

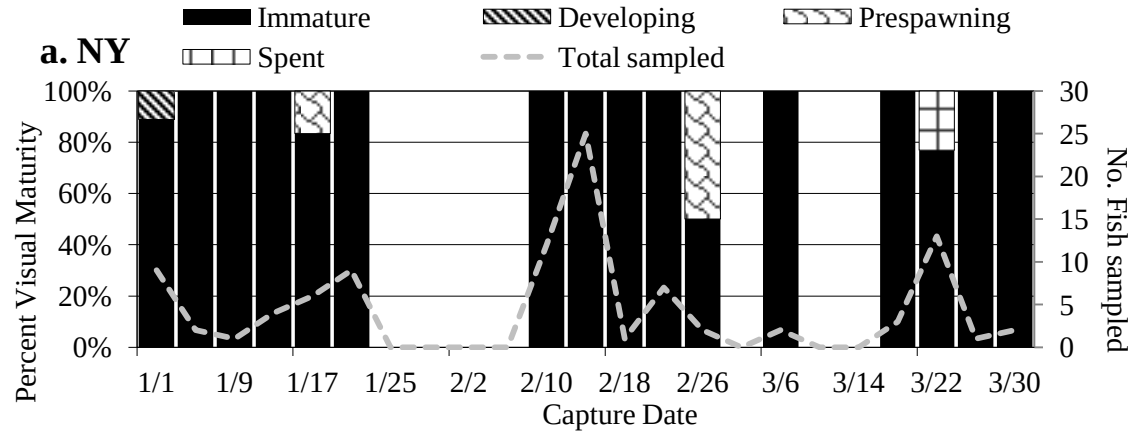
through the spawning season. Data for each histology category were charted by gross anatomical maturity assignments. Solid bars show agreement between histology and gross visual maturity. Stippled bars are used to display misclassified ovaries by the stages into which they were incorrectly classified.

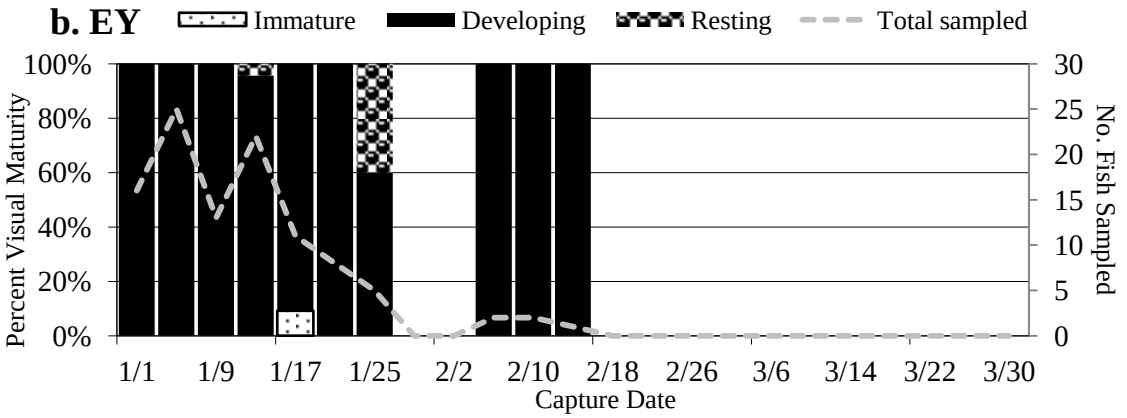
The patterns shown in Figure 1.4 show the fluctuations in sampling the various stages through the season, with the exception of ovaries with NY oocytes. Sampling levels remain consistent over time for NY stages or reserve fund oocytes which do not mature through the season as the fishery takes fish prior to the age of spawning. Ovaries with oocyte structures of EY, MY, LY were more frequently classified correctly when levels these structures were highest in the ovaries. Ovaries with EY oocytes were misclassified as immature and resting stages at low rate early in the spawning season. Ovaries with MY oocytes had few misclassifications early in the season but were misclassified as prespawning stage extensively starting in mid-spawning season. Ovaries with LY oocytes had a high rate of misclassification as developing stage early in the season but this decreased later in the season. Ovaries with HYD oocyte were misclassified as prespawning stage at mid-spawning season and as spent stage later in the season.

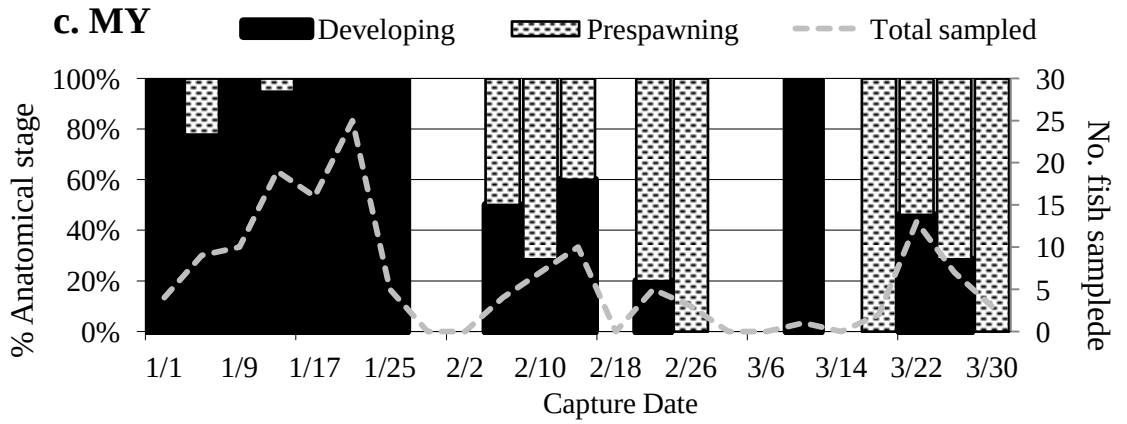
In most cases early misclassifications were incorrectly identified as an adjacent less mature stage and later misclassifications were the adjacent more stages. Ovaries with LY and YC were an exception to this pattern with misclassification of developing stage later in the season in samples with both structures.

Charts for POF and TKNY are not shown because sample sizes were very small. Misclassification of POF structures did not show variation over time, while TKNY showed a range of misclassification as immature and spawning stage later in the season.

Figure 1.11 Patterns in the comparison of gross anatomical assignment and oocyte structures determined through histological evaluation from the same fish are charted over time. The solid black bars represent correct classifications and the stippled bars show misclassifications identified through unique stipple patterns. Graph bars represent the daily percent of anatomical stage assignments and align with the left axis. The gray dashed line represents the number of fish sampled per day and aligns with the right axis.


a. NY
Immature
Developing
Prespawning
Spent
Total sampled
Percent Visual Maturity
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
No. Fish sampled
1/1
1/9
1/17
1/25
2/2
2/10
2/18
2/26
3/6
3/14
3/22
3/30
Capture Date


b. EY
Immature
Developing
Resting
Total sampled
Percent Visual Maturity
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
No. Fish Sampled
1/1
1/9
1/17
1/25
2/2
2/10
2/18
2/26
3/6
3/14
3/22
3/30
Capture Date


c. MY
Developing
Prespawning
Total sampled
% Anatomical stage
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
No. fish samplede
1/1
1/9
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2/10
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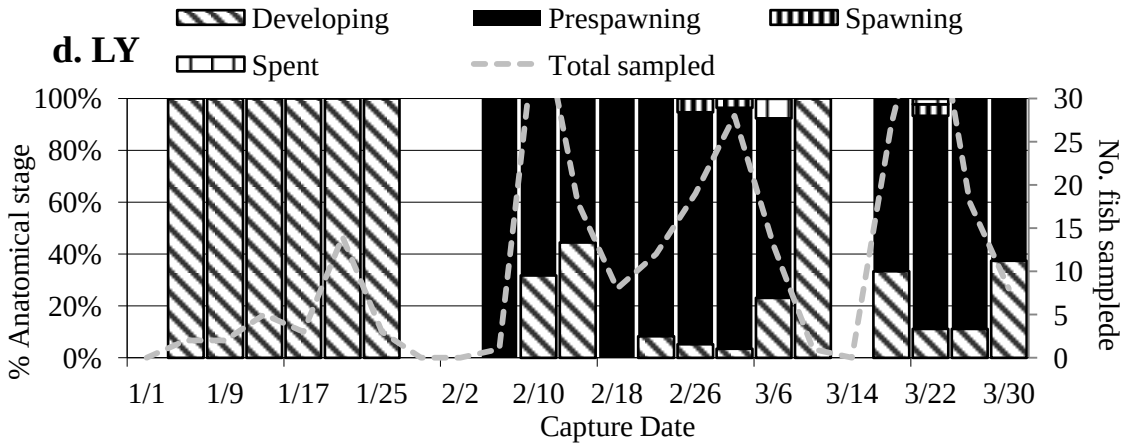
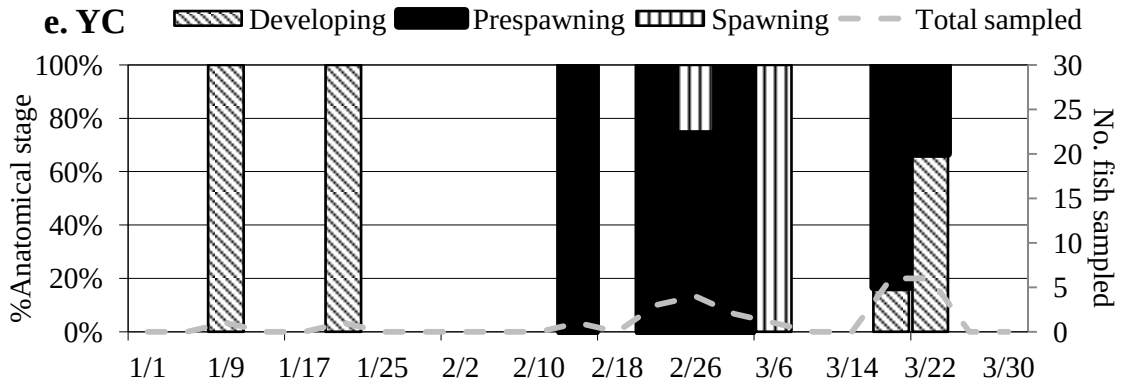
Capture Date


d. LY
Developing
Prespawning
Spawning
Spent
Total sampled
% Anatomical stage
No. fish samplede
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
1/1
1/9
1/17
1/25
2/2
2/10
2/18
2/26
3/6
3/14
3/22
3/30
Capture Date


e. YC
Developing
Prespawning
Spawning
Total sampled
%Anatomical stage
No. fish sampled
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
1/1
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3/14
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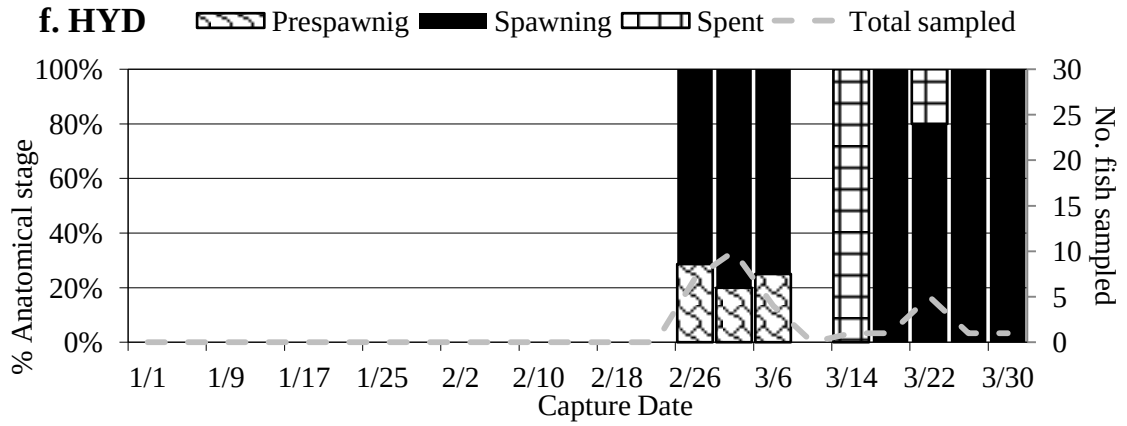
3/30


f. HYD
Prespawnig
Spawning
Spent
Total sampled
% Anatomical stage
No. fish sampled
100%
80%
60%
40%
20%
0%
30
25
20
15
10
5
0
1/1
1/9
1/17
1/25
2/2
2/10
2/18
2/26
3/6
3/14
3/22
3/30
Capture Date

Discussion

Where misclassifications occurred, the error was within +/- one maturity stage 88% of the time. Much of the misclassifications may have occurred with ovaries in transition between stages where the gross anatomical appearance presented characteristics of two neighboring stages. Variations in the interpreting the key can account for these misclassifications. Estimating ovary maturity requires the delineation of advancing stages of development between samplers. Because oocyte development occurs on a continuum, delineation between levels of maturation can be subjective.

The misclassification rate for observer collections was twice the rate for the FIT researchers. This was likely due to the increased collaboration and sampling experience of the FIT group. Observers work independently and may have varying interpretations of the key descriptions, such as those describing ovary color and size of ovary relative to the abdominal cavity. Maturity assessments are made by comparing ovary characteristics to the photos and descriptions in the key and while the text and photographs define stage characteristics, the assignment of a maturity stage to an ovary in transition between two stages is left to the judgment of the sampler as to which stage best describes the ovary. Post-cruise observer comments often focused on the difficulty in assigning maturity stages to ovaries in transition, additional comments were made concerning the lack of images to fit the appearance of early developing ovaries.

Current convention recommends using histological staging to validate gross anatomical assignments (West 1990). However, when examining oocytes histologically, early changes in development can be detected in some oocytes. These early changes in a small number of oocytes may not affect changes in the gross anatomical appearance of the ovary. Rather, it is more likely that the changes occur as the majority of the oocytes reach a certain level of maturation within the ovary. Some level of accumulation of a certain oocyte category may be necessary before these changes are realized

macroscopically. Therefore, staging the ovary based on the most advanced oocytes observed, may overestimated the maturation level.

Misclassification for immature stage (NY)

A comparison of the maturity stage assignment to the oocytes structures present in ovaries from the FIT cruise data show strong agreement between NY structures and the assignment of the immature stage. However, comparisons of these assignments for the observer collections present a high rate of misclassification as developing and resting stages. This may be due to increased observer sampling of immature and resting stage fish. Observers may have encountered more fish in the secondary growth phase, prior to the development of the CA which are difficult to differentiate between immature, early developing, or resting. Histological evaluation will show a lack of yolk development (NoYolk) in each of these cases but does not allow for differentiation between these oocyte structures.

The immature category describes a young fish that have not previously spawned. Resting stage fish, those that have spawned previously and revert to the resting stage after the spawning season, that fail to mature during the current season are most often confused with the immature fish. Differentiation of these stages through histological assessment is not definitive as there are no variations in the oocyte structures between these stages. The presence of very old POF remnants may be seen or, if the sample contains a portion of the ovary wall, a thicker ovary wall may identify a specimen as resting rather than immature. The distinction between immature and developing or resting is important if maturity data are to be used for age or length at 50% maturity studies and presents an ongoing challenge for fishery managers. Because observers assess maturity through the spawning season, the presence of No Yolk, whether immature, early developing, or

resting are readily differentiated from the rest of the maturating stock with maturity key collections.

EY and MY (developing) and LY and YC (prespawning)

The majority of misclassifications occurred between developing and prespawning stages. As with estimating vitellogenenis stages macroscopically, the microscopic estimation of the size and abundance of yolk droplet can also be subjective. Vitellogenesis within oocytes progresses on a continuum with changes occurring slowly over time. Therefore misclassification of this stage may be due, in part, to the difficulty of delineating the variation in yolk accumulation and nucleus migration stages in the oocytes. Nuclear migration is often difficult to assess and may depend on the specimen orientation on the slide. The degree of migration appears independent of yolk droplets advancement in some individuals. Therefore, these landmarks are often less clear on both scales. Ormseth (2007) measured egg size as an indication of parental investment in offspring survival and found that egg sizes varied with the condition of the mother. Therefore, the use of the relative size of oocytes or the quantity of yolk droplets size may less reliable in delineating oocyte stages histologically.

Table 1.5 indicates that misclassification between vitellogenesis stages occurred most often with ovaries containing Late Yolk wrongly identified and the less advanced developing stage. The misclassification of ovaries that contain MidYolk oocytes also occurs but at a lesser (25%) rate. This trend suggests that the gross anatomical key may underestimate the level of maturation for vitellogenesis stages.

HYD (spawning) and POF (spent)

Misclassification of fish with prespawning stage oocytes as spawning can occur when samplers apply too much pressure to the abdomen, causing ovary tissue to rupture and the release non-hydrated oocytes. Additionally, ovaries with fully hydrated oocytes may be

assessed prematurely as spent stage due to the effects of gear-related trauma associated with capture. Aboard research cruises employing pot gear, fish were often seen releasing eggs as the pot was being recovered. These fish were identified post-rupture as spent stage upon reaching the sampling tables (personal observation).

TKNY (resting)

Gross visual maturity keys for other gadoids such as walleye pollock (Stahl 2004) include a seventh stage located between immature and developing to account for maturing, but pre-vitellogenesis fish. The addition of a seventh stage to identify developing fish that are in a pre-vitellogenesis state may help to clarify the immature and resting stages. This seventh stage would be useful in identifying individuals that begin to develop, but fail to spawn during the year. This stage may provide additional insight on fish condition in response to environmental changes.

The use of maturity stage assignments for stock assessment parameters

Stark (2007) analyzed the geographic and seasonal variations in maturation of Pacific cod in the GOA and BS through histological processing of ovary specimens and concluded that macroscopic staging was ineffective in differentiating ovaries with early developmental structures and post-ovulatory follicles. While these limitations are present in the use of the maturity key, this does not negate the value maturity key assessed maturity. Stark cites Hunter's argument that early developing stages are likely to mature within the season for multi-batch spawning fish and should be accounted for in stock assessment calculations. Because Pacific cod are synchronous, single batch spawners, if these early developing fish occur during the peak of the spawning season, I maintain that they are out of sync with the rest of the population and should not be included as members of the spawning population. And because observers are able to assess maturity later in the season when differentiating between immature and early maturing and fish is

less problematic, misclassifications are less likely. This analysis shows that, out of 182 fish with early yolk oocytes assessed by observers, 17, or 9% were incorrectly identified as immature stage fish while none were misclassified as resting or spawning stages. The occurrence of these early stages for Pacific cod during the peak of the season was rare and unlikely represents a later fall spawning season as no spawning activity has to date been identified in the Bering Sea in the summer or fall.

Because of the broad temporal scope of the maturity collections these data are appropriate for tracking spawning stock for this species. Length at 50% (L50) maturity estimates were calculated using observer gross anatomical maturity data from 2004-2009, providing a range between 55 and 60 cm (Logerwell and Neidetcher 2009). These results were consistent with the estimate for L50 of 57.9 calculated from the 2003-2005 histological specimens collected during the FIT cruises (Stark 2007). Stark (2007) also identified variation in L50 between the Aleutian Islands and the Bering Sea. Observer maturity data collection from regions across the BSAI will enable researchers to track changes in L50 continually, along with variation in climate and stock conditions.

Conclusion

Gross anatomical maturity keys provide an inexpensive and easily applied method to estimate reproductive maturity. Comparison of the maturity key data to individual oocytes assessed histologically provides misclassification rate for key developers collaborating over stage delineations was 22%, while fishery observers with less experience evaluating maturity stages and working independently had a misclassification rate of 43%. Of the misclassifications, 88% of the maturity assignments occurred between adjacent stages and were likely, for at least some of the misclassified stages, a result of ovaries in transition and exhibiting characteristics of two of the maturity stages. Although misclassifications were high with developing and prespawning stages; the key misclassifications are more often in the direction of earlier maturation stages rather than

more advanced staging. However, misclassification rates suggested that 18% of ovaries identified as spawning stage in spawning stage cod contained the less mature vitellegenesis stages oocytes. Use of the maturity key data to estimate spawning location may include some fish in later Yolk stages and may just beginning to hydrate. While the duration of the later yolk stages in unknown, it is likely these fish are very close to spawning. In chapter 2 of this thesis I describe spawning locations using prespawning and spawning stages. Because vitellegenin stage misclassification are more conservative, i.e., ovaries containing Late Yolk and Yolk Coal were more often misclassified as developing stage rather than Early Yolk and Mid Yolk misclassification as prespawning (by 25%), key assignments would most often fail to identify a spawning location rather than incorrectly identify a location where spawning occurs.

These results also suggest caution in using histological estimation maturity stages to validate the gross anatomical maturity key. Care should be taken in staging ovary maturity by evaluating structures at the microscopic level. Initial work should include a calibration process to insure which microscopic structures align with shifts in gross characteristics at the macroscopic level. Histological structures such as vitellogenesis, hydration, and post-ovulatory follicles demark stages in ovary maturation, though at what point these changes result in changes at the gross visual scale should be considered.

One recommendation for the improvement of the key interpretation is to include a description of the ovarian maturation process in the observer training material. Through a clearer understanding of the process, observers may improve their use of the maturity key. Also, the inclusion of an early developing stage may alleviate some confusion for fish in transition between immature and developing stages fish. Increasing sampling from non-spawning season will also fill gaps currently present in the maturity key.

CHAPTER 2: SPATIAL AND TEMPORAL SPAWNING PATTERNS OF PACIFIC COD IN THE EASTERN BERING SEA AND ALEUTIAN ISLANDS REVEALED FROM GROSS ANATOMICAL MATURITY DATA

Introduction

Marine fish recruitment is influenced by both biological and physical processes operating at changing spatial and temporal scales, and is known to be highly variable (Hollowed et al. 2002). Researchers seek to identify spawning patterns as a component of this complex process (Bailey et al. 2005). Knowledge of spatial and temporal patterns of spawning provide researchers the opportunity to link spawning to features or conditions of the spawning locations that may be important to spawning fish or the survival of their offspring. This knowledge allows the evaluation of recruitment success over changing conditions at these sites both on small-scale temporal and spatial changes and larger oceanic and decadal ranges. Additionally, knowledge of spawning areas allow researchers to assess spawning migrations, to identify early life stage distribution and nurseries where young fish develop, and to provide a general understanding of population structure (Hjort 1926, Sinclair and Iles 1988). Therefore, knowledge of the distribution and spawning site locations, along with spawning phenology may provide insight into spawning and recruitment strategies, and is essential to the investigation of interactions between a species and its environment.

Though the mechanisms determining timing and location of spawning are poorly understood (Cury 1994), this process is assumed to be linked, through natural selection, to ecological conditions beneficial for early life survival (Leggett 1985). The spawning site provides the initial conditions under which early life stages develop. Spawning site features such as topography and bathymetry set the landscape in which these processes unfold (Ciannelli et al. 2007). Meteorological and oceanographic cycles interacting with site features determine the physical conditions present at the time of spawning.

Giesel (1976) describes optimal reproductive timing as a vital part of life history strategy. Cushing (1969) suggests that spawning phenology is linked to the timing of processes at the spawning sites that are most beneficial to developing larvae, such as high concentrations of prey, or in dispersal potential of eggs and larval stages to estuarine nurseries or protected bays. Sabatés et al. (2007) points to the structure of the bathymetry, the bottom type, and the mechanisms controlling primary production as determining the location of spawning, while physical processes such as frontal systems and associated currents, storm mixing, and water column stratification determine the distribution of eggs and larvae.

From these previous works on spawning distribution in marine fish, it is clear that spawning phenology and geography can be both geographically and hydrologically fixed. The degree to which one set of variables prevails over the other depends on the species and is likely to be a function of life history strategy. Species phenotypic plasticity may allow tolerance for changes in environmental conditions, though optimal success may be tied to processes that occur in geographically fixed locations.

Reproductive strategy, according to Cury (1994) includes a mechanism for finding a mate and for linking an individual to a reproductive environment. Cury (1994) describes the identification of spawning sites as including the detection of cues (timing) and clues (location) associated with conditions or locations appropriate for spawning, and suggests that these spawning environmental triggers are often a result of successful recruitment over prior generations.

The objectives of this chapter include the identification of spatial and temporal spawning patterns found by mapping data characterizing the reproductive maturity of Pacific cod (*Gadus macrocephalus*). Maturity data assessed by observers monitoring catch aboard commercial fishing vessels in the eastern Bering Sea and Aleutian Islands (BSAI) from

2005 to 2007 are included in this thesis. The spatial and temporal scale of the observer maturity collections allowed for a comparison of spawning patterns from fished locations across the eastern Bering Sea and Aleutian Islands (BSAI). Multiple collections over three consecutive years allowed for comparison over regional scales and within local scale over the study years. In this chapter, the geographical locations of spawning are described, as are the generalized oceanic conditions at the time of spawning. The discussion includes speculation on what these patterns may imply about Pacific cod recruitment strategy as well as stock structure.

The next section provides background information for Pacific cod; what is known regarding spawning behavior, early development, and environmental constraints for Pacific cod survival. An additional section includes a general description of oceanography and environmental variables for the BSAI. This information, primarily concerning hydrographic features, is included to provide a background for more detailed site-specific conditions that may be related to Pacific cod spawning patterns.

Pacific cod biology

Pacific cod occupy near-bottom waters (Bakkala et al. 1984, Nichol et al. 2007) along the continental shelf and upper slope at depths of 80 - 260 m, less frequently to 500 m. Tagging studies have identified spring spawning and summer feeding migrations (Shimada and Kimura 1994), and have indicated site fidelity to individual spawning locations (Gustafson et al. 2000, Shi et al. 2007). Pacific cod form large spawning aggregations in the late winter and early spring (Shimada and Kimura 1994). A single batch of eggs is released annually and oocyte development is synchronous (Mecklenburg et al. 2002).

Pacific cod eggs have a greater density than sea water and sink upon release during spawning (Thomas 1963). Because eggs are demersal and slightly adhesive, their distribution is presumed to be limited to the spawning grounds (Yamamoto and Nishioka 1952, Laurel et al. 2008). Pacific cod eggs incubate on bottom substrate (Yamamoto and Nishioka 1952, Forrester and Alderdice 1966) for approximately 19 to 22 days (Laurel et al. 2011) and then larvae rise to the surface of the water column upon hatching where currents may transport them to near-shore nursery areas (Hurst et al. 2009, Laurel et al. 2009). Typical bottom substrate such as coarse sand, gravel, or rocky outcroppings may be preferable for adhesion and aeration (Hurst pers. comm.). Diel vertical migration patterns are seen within 28 days of hatch (Hurst et al. 2009) suggesting larvae may have some control over their dispersal patterns, moving up and down in the water column at will.

Pacific cod in early life stages have been observed in variety of habitats, both close to shore and in open waters. For example, Walters (1984) reported Pacific cod larvae at the bottom of a shallow inlet in Washington. Rugan and Matarese (1988) found that larvae were widely distributed over the continental shelf near Kodiak, with few caught seaward of the 200 m isobath. More recent near-shore assessments in the Gulf of Alaska Kodiak region have identified Pacific cod along coastal regions serving as nursery areas for young juveniles, with age 0 cod utilizing macroalgae and eelgrass beds during younger ontogenetic stages (Abrookire et al. 2007, Laurel et al. 2009). In the eastern Bering Sea, juvenile Pacific cod are thought to inhabit near-shore waters during their first year, but then move to deeper water as they mature (Bakkala, 1984). Larval fish assemblages including Pacific cod are reported to occur along the outer shelf and in the northeastern area near Unimak Island inshore of 100 m isobaths (Siddon et al. 2011). Duffy-Anderson et al. (2005) speculated that onshore currents may serve to move larval fish onto the continental shelf.

Species tend to evolve to optimize their fitness for a given set of circumstances and environmental variability must be factored into coping strategies. For many broadcast spawners, such as Pacific cod, parental investment is low and energy resources are directed to high levels of fecundity (Winemiller et al. 2005). Eggs are released into the environment and yield high recruitment when conditions are favorable, perhaps on seasonal to even decadal scales (Winemiller et al. 2005). Offspring are provided with few resources and must find suitable habitat and food quickly to survive. To be successful, the spawning location must include a means of obtaining needed resources for offspring. The cues and clues directing spawning populations must account for these future conditions.

Temperature is considered an important constraint for many marine species. Adult Pacific cod are temperature sensitive and can inhabit waters within the range of -1.5 to 18◦ C, but are best adapted to ranges between 2 and 10◦ C (Moiseev 1953). They are found to avoid the Bering Sea cold pool waters (a mass of cold water over the shelf region that varies in size and is related to the ice extent) ranging from 0 - 2◦ C in the summer (Ciannelli and Bailey 2005, Mueter and Litzow 2008). Laboratory studies have shown that temperature is an important environmental constraint on the size, growth, and endogenous reserves of Pacific cod larvae (Laurel et al. 2008). Temperature is suggested to be a key factor in successful egg development, with incubation success found to occur between 2.5 and 8.5◦ C (Alderdice and Forrester 1971). Laboratory studies have found that, although larvae do hatch at 0◦, hatch rates were poorer at this temperature. Growth rates were highest, for both pre-hatch and pre-feeding larvae at temperatures between 0 and 4◦ C (Laurel et al. 2009). Additionally, colder temperatures may extend the number of days before hatch. Therefore, spawning sites are likely centered on areas where favorable incubation temperatures exist (Westrheim 1996).

Juvenile Pacific cod are also shown to perform diel vertical migration in response to environmental factors including temperature and the presence of food (Davis and Ottmar 2009). For Pacific cod, Alderdice and Forrester (1971) found that ocean conditions such as temperature, salinity, and oxygen levels provide localized constraints in development, while hydrographic features such as ocean scale currents, fronts, eddies, and water column stratification may influence the distribution of early-life stages and their prey**.**

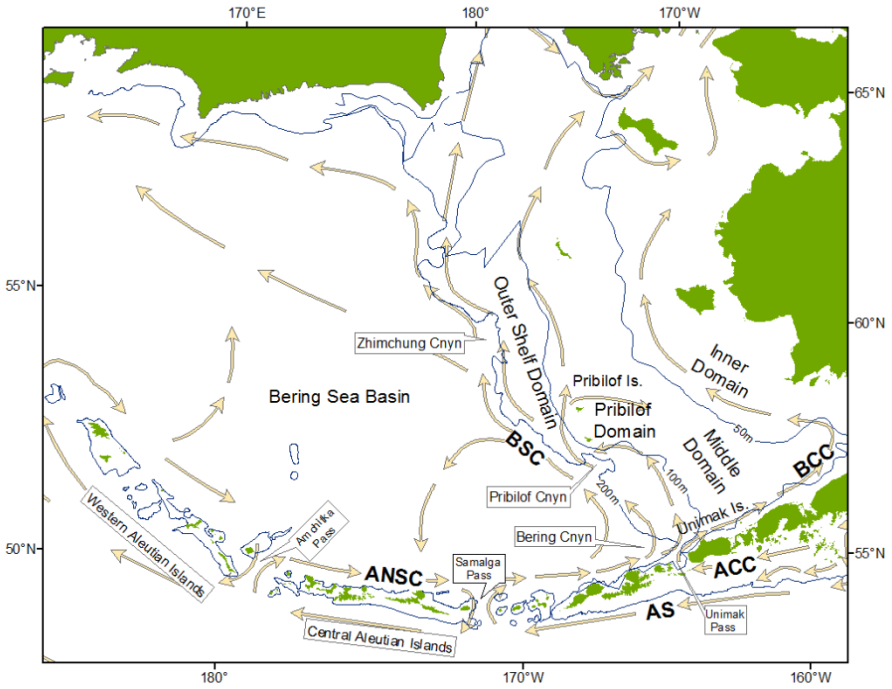
The timing of spawning for temperate fish species is thought to coincide with conditions beneficial to early life stages (Cushing 1969). If alignment with seasonal fluctuations in prey abundance drives the phenology of spawning, we might expect to see increased primary production associated with spawning and early life development. The timing of primary production in the Bering Sea is dependent on the mechanism of surface water stratification (Stabeno et al. 2010). Early ice-associated blooms occur along the Middle and Outer Domains in years with high ice cover; while open-water blooms, in low ice years and along the Aleutian Chain, typically occur later in the season (Luchin et al. 1999) and rely on decreased storms and increased solar illumination to warm surface waters. However, early blooms may occur in temperatures too cold for utilization by zooplankton species. This could result in a decoupling in the food web, meaning that early blooms may not be particularly beneficial to larval Pacific cod (Napp et al. 2002). Laurel et al. (2011) found a reduction in metabolic rate during colder conditions, mitigating the absence of prey for longer time periods, while during warmer temperatures cod were more vulnerable to longer periods without prey. Laurel et al. (2011) suggested that a later spawning date, matched to a later bloom, may be the most advantageous for cod.

General Oceanography

The Bering Sea (Figure 2.1) is bordered along the southeast by the porous volcanic ridge that comprises the Aleutian Island Chain, which separates the North Pacific Ocean and

the Bering Sea. The southeastern Bering Sea is a semi-enclosed, subarctic sea comprised of a 500 km-wide continental shelf to the northeast and a deep-water basin to the southwest. The Bering Sea Slope dissects the Bering Sea diagonally from the southeast to the northwest into a shallow shelf and a deep-water basin. From the basin, deep-sea canyons intersect the slope. The southernmost canyon, the Bering Sea Canyon, breaches the slope at the intersection of the shelf and the Aleutian Island arc and Unimak Pass. The Pribilof Canyon cuts into the slope just south of the Pribilof Islands, while the Zhemchug Canyon is situated over 200 km further along the slope to the northwest.

Figure 2.1: A schematic of the upper ocean flow in the Bering Sea during the summer months. Depths are in meters (modified from Schumacher and Stabeno 1998; Stabeno et al. 1999). Bering Sea currents interact with water column hydrography to differentiate physically and biologically distinct domains in the Southeast Bering Sea.



Water enters the Bering Sea through a series of passes along the Aleutian archipelago and exits primarily through the Bering Strait. Four primary currents flow through the southeastern Bering Sea and the Aleutian Islands. Flow from the Alaska Coastal Current

(ACC), fed with freshwater from coastal rivers along the northern Gulf of Alaska (GOA), enters through the more eastern passes. This current flows west along the southern side of the Aleutian Peninsula. The Alaskan Stream (AS), a western boundary current and the eastern part of the North Pacific subarctic gyre, also follows the coastline of the western GOA. The AS flows seaward of the ACC and enters the Bering Sea through the more western deep-water passes, providing heat and high nutrient levels along its trajectory (Ladd et al. 2005). The Aleutian North Slope Current (ANSC) flows northeast along the north slope of the Aleutian Islands, and is modified by water flow from the ACC through the Aleutian passes. The Bering Slope Current (BSC) is a northwestward flowing extension of this current and follows the shelf break of the eastern Bering Sea (Shumacher and Reed 1992).

Bering Sea water column hydrography

Hunt et al. (2008) divide the Bering Sea shelf into seven domains separated along isobaths and defined by discrete circulation patterns. These domains include three shelf domains: 1) a Coastal Domain is situated shoreward of 50 m isobaths, 2) the Middle Domain is contained between 50 and100 m isobaths, and 3) an Outer Shelf Domain lies between 100 and 200 m isobaths. The continental 4) Slope Domain separates the shelf from the deep-water 5) Basin Domain. The recirculation of tidal flow, the addition of on-shelf flow due to proximity to the shelf edge and the Pribilof Canyon, and the effects of variable flow from the BSC result in a unique hydrology surrounding the 6) Pribilof Islands Domain (Kinder et al. 1983, Brodeur et al. 2002). Finally, the area north of Unimak Island combines on-shelf flow from the near-shore bathymetry to differentiate these areas as the 7) Unimak Island Domain.

The dynamic system formed by shelf-slope fronts is often characterized by strong gradients in temperature, salinity, and flow velocities (Fedorov 1986). Fronts and

associated currents often produce instabilities such as eddies, meanders, and intrusions that vary on spatial and temporal scales (Wang et al. 1990). Frontal zones produce barriers that often serve to accumulate and condense (Iles and Sinclair 1982), while eddies and meanders may serve to disperse nutrients, fish larval stages, and plankton (Sabatés et al. 2004).

Aleutian Islands hydrography

The structure of the flow through the passes is dominated by tidal currents (Stabeno et al. 2002b). Stabeno et al. (2005) suggests that water masses from the Pacific and the deep Bering Sea Basin mix in the passes due to abrupt topography and the constant motion of tidal forces. Passes along the Aleutian Islands range from narrow with shallow sills in the east to wider with deeper sills in the west. Typically, the water column is well mixed within the passes (depending on the depth and width of the pass); however, as water moves away from the mixing forces, and especially on the lee sides of islands, mixing forces ease (Ladd et al. 2005). Fronts separating mixed and stratified regions in the Aleutian passes are altered with seasonal cycles and climate changes. For instance, fronts shift and strengthen as seasonal temperatures warm in the early spring and their patterns may vary as yearly temperature regimes change.

BSAI water temperature

Ocean temperature in the BSAI is influenced by warmer water entering the Bering Sea from the Pacific through the Aleutian passes. From January to June hydrographic fronts develop where warm Pacific water entering from the Aleutian Passes funnel between the shelf and the deep basin along the slope (Stabeno et al. 2005). Heat exchange also occurs with tidal currents bringing warmer, deep-waters on-slope (Stabeno et al. 2002a, Kinney et al. 2008). Ocean temperature is also climate driven, with decreases in air temperature

and increased ice formation resulting in temperature changes throughout the water column (Mueter and Litzow 2008).

Sea ice formation and advection

Sea ice is a dominant feature of the northern Bering Sea. Climate patterns influences the level of cold northerly winds that cool shelf waters and result in ice formation along the shelf. Northerly winds carry sea ice south until it reaches warmer water and melts (Niebauer et al. 1999), creating a dynamic ice edge that shifts with changing weather patterns. During high ice years, the ice edge extends to the shelf break and toward the southeast corner of the shelf (Stabeno, 2002a). Ladd and Stabeno 2012 suggest that ice melt plays an integral part in water column stratification during high ice years. Mechanical mixing, through tidal flow and wind-generated turbulence in the fall and winter, results in a homogeneous water column over the Bering Sea shelf. The relaxation of wind events, and either melt-water accumulation in ice-cover areas, or warming by thermal radiation in open water, results in surface water isolation. Solar isolation and the easing of winter storms drive water column stratification in the Aleutian Islands.

Methods

Data Collection

Observers with the Alaska Fisheries Science Center (AFSC), Fisheries Monitoring and Analysis Division (FMA) assessed reproductive maturity using a gross anatomical maturity key in Pacific cod commercial catches taken in the BSAI from 2005 to 2007. Observers sampled catches from Pacific cod directed fisheries, and from Pacific cod caught as incidental catch during directed fisheries for other species. The capture gear included trawls, longlines, and pots. Prior to departure, observers were trained in the use of the maturity key. The maturity key has been validated by comparing a subset of the fish assessed for gross anatomical maturity to histological analysis of ovary maturity for

the same fish. Detailed collection methodologies are described in Chapter 1 of this thesis.

Mapping and analysis of observer macroscopic maturity data

Using ESRI Spatial Analysis software, the catch locations for observer-assessed gross anatomical maturity were plotted to display the spatial distribution of spawning in the BSAI. Rules of confidentiality apply to protect commercial fishing information. To avoid revealing catch locations for any given fishing vessel, haul locations were binned according to a 20x20 nautical mile grid system. Data in the grid cells with fewer than three vessels were not plotted.

For these maps, the capture location of fish in spawning and prespawning stages is plotted as a proxy for spawning locations. A comparison of gross anatomical maturity staging and the maturity estimate of individual oocytes within these ovaries suggest there were misclassifications with the majority of specimens classified as prespawning but containing less advanced yolk stages. The results suggest that mapped stages may identify locations as spawning that may include less mature fish for 15% of the mapped data. Prespawning and spawning stages are color-coded to identify locations where these stages were observed. Cells were marked for spawning if spawning stage fish were observed in the cell. Cells were marked as prespawning where prespawning stage, but no spawning stage fish were observed. Immature, developing, spent, and resting stages are color-coded identically to identify locations where observers assessed maturity but did not observe spawning or prespawning stage fish.

*"*Hot Spot" maps

The spatial distribution of concentrated spawning was analyzed using observer-assessed gross anatomical maturity from times when samples showed a high percent of spawning. To look at locations of high percent spawning, the locations of days with high percent spawning were isolated from the rest of the data. The percent of spawning stage

assessments per day were calculated by dividing the number of spawning stage assignments by the total number of ovaries examined each day from January 1 through April 30. Days with 15 percent and greater spawning stage assessments were identified as high percent spawning days, or "hot days".

$$\% \text{ spawning stage assessments per day} = \frac{\text{\# of spawning stage ovaries, per day}}{\text{\# of ovaries examined, per day}} * 100$$

The haul locations for spawning stage fish during hot days (days with spawning stage observations >15%) were plotted to identify locations of concentrated spawning. To preserved fishing vessel anonymity, hot day catch locations were binned in accordance with the State of Alaska Groundfish/Shellfish Statistical Areas for the Bering Sea and the Aleutian Islands. Statistical areas with less than three vessels per area were not displayed. The percent spawning was recalculated for each statistical area and displayed through color coding. The number of spawning stage fish sampled per area is displayed on the map, with a cross pattern used to identify areas included as hot days, but where no spawning fish were identified.

Phenology Charts

Phenology charts provide an effective way to display temporal patterns in spawning, and to compare these patterns among years and regions. For these charts, observer-assessed maturity data were grouped into pre-spawning and post-spawning categories. The pre-spawning category includes the developing and prespawning stages, while the post-spawning stage includes the spawning and spent stages. The misclassification rate for the combined pre-spawning and post-spawning stages calculated through the comparison of gross anatomical staging to histological analysis of the oocytes within maturity assessed ovaries equaled 3% (Table 1.5 Chapter 1). Fish classified as immature or resting were

omitted from these analyses. Line charts were constructed from the percent of pre-spawning and post-spawning categories over time. Collections from January 1 to April 30 were pooled into 5-day intervals to reduce noise and determine the general pattern over the spawning season. The beginning of the spawning season was defined for this analysis as post-spawning levels of at least 20%. Levels between 40 and 60% were considered mid-spawning and post-spawning threshold of 80% was considered the end of the spawning season.

Results

Observer-assessed maturity collections

Between 2005 and 2007, observers determined the maturity status of 37,268 female Pacific cod (Table 2.1). Sampling levels were highest during 2005, remained high in 2006, but dropped with fewer observers participating in the project in 2007. While the proportion of the maturity stages was similar for the three years, a lower fraction of spawning and spent stages fish was observed during 2006. In 2006 the number of spent stage identifications was considerably lower than the other two years.

Table 2.1 Gross anatomical maturity stages for female Pacific cod assessed by FMA observers from January 1 – April 30 aboard commercial fishing vessels in the Bering Sea and Aleutian Islands. The parentheses include the percent of each maturity stage per year. No. Obs. = number of observers

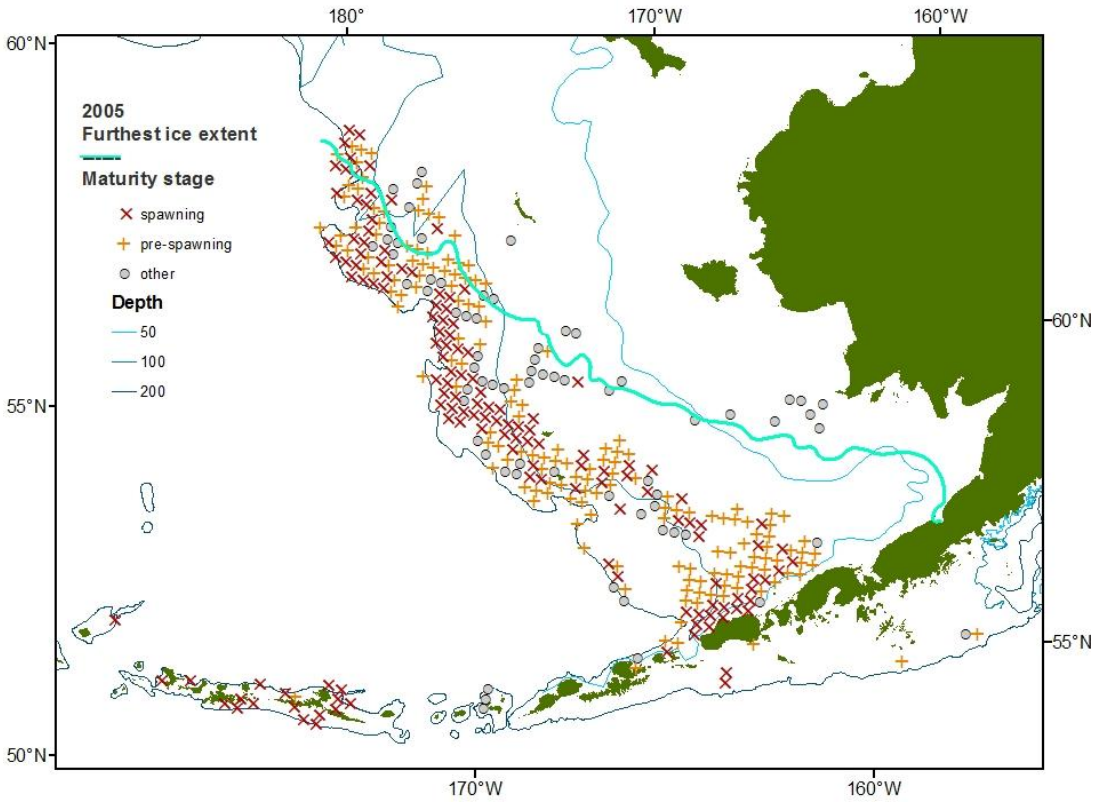
Year	No. Obs	Immature	Developing	Prespawn	Spawning	Spent	Resting	Total
2005	76	3352 (23)	2947 (20)	4875 (33)	857 (6)	1285(9)	1385(9)	14703
2006	81	3505 (26)	3080 (23)	4927 (37)	838 (6)	408 (3)	655 (5)	13413
2007	58	2005 (22)	2104 (23)	3034 (33)	470 (5)	894(10)	645(7)	9152

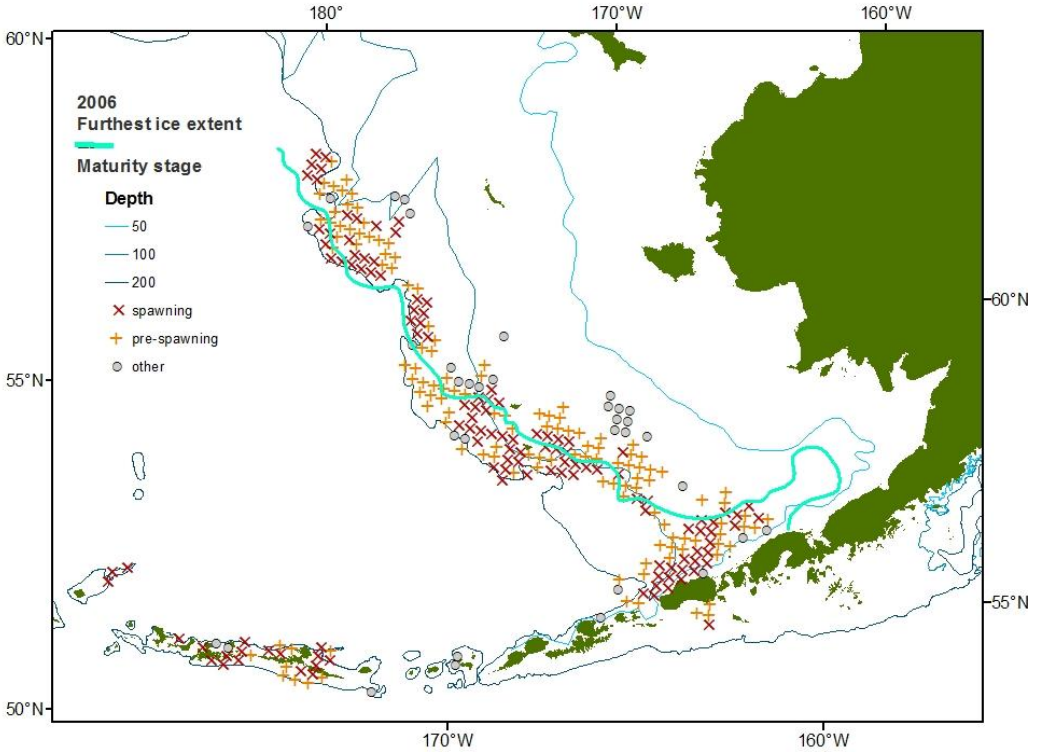
Spawning locations

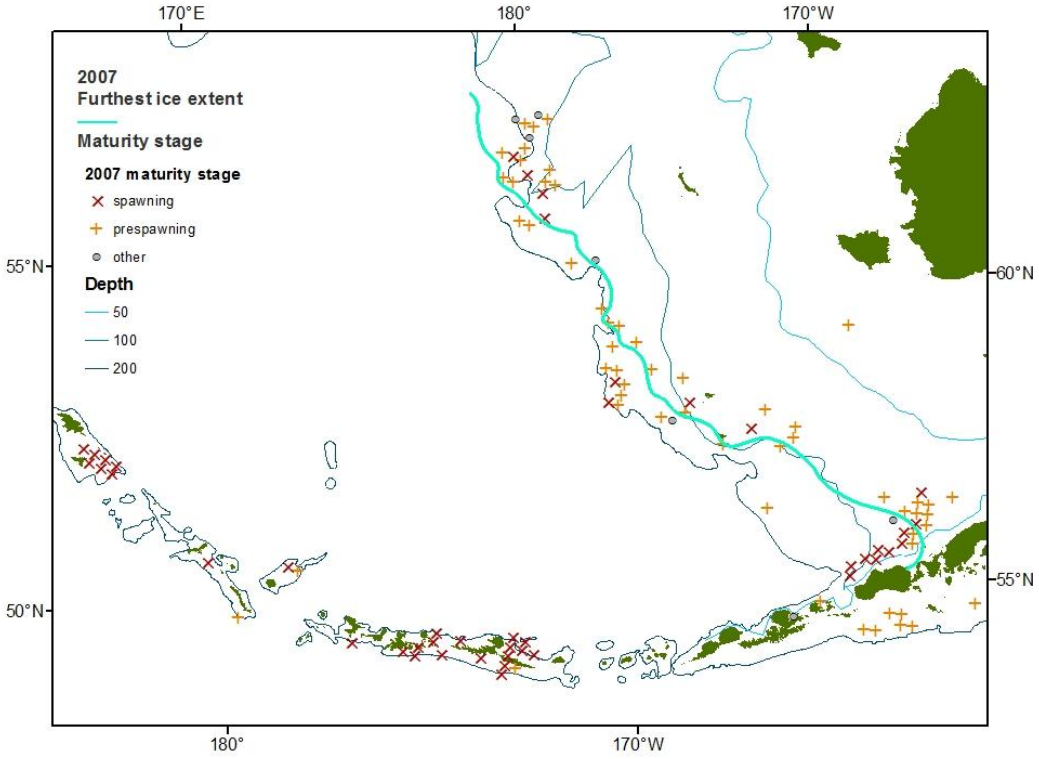
Annual maps of the capture locations of prespawning and spawning stage female Pacific cod identified spawning locations along the eastern Bering Sea Northern Outer Domain (100 to 200 meters in depth). Additional areas were identified in the eastern Aleutians from Unimak Pass to Amak Island, the central Aleutian Islands from Amlia Island to Tanaga Island, and the western Aleutian Islands from Petrel Bank to Attu Island (Figure 2.2). Observer sampling effort in 2005 and 2006 identified widespread spawning along the northern outer shelf from southeast of the Pribilof Islands to west of St Matthew Island. Sampling effort was similar in 2007 and though prespawning stage fish were observed, fewer spawning stage fish were observed along the northern outer shelf in 2007. Observed spawning sites along the Aleutians were fairly consistent for all three years.

Spawning identified along the Bering Sea outer shelf occurred primarily between the 100 and 200 meter isobaths. Because observers sample aboard commercial fishing vessels, sample locations were dependent on where the vessels fished. Spawning may occur at deeper depths off the outer shelves, beyond the reach of fishing gear; however, shallower fished locations (less than 100 m) along the middle and inner shelf showed few prespawning and spawning stage fish, suggesting that cod prefer deeper spawning sites.

Figure 2.2 Distribution of prespawning and spawning stages of female Pacific cod from observer-collected gross maturity data in 2005-2007 from January-April. Commercial fishing information is protected by rules of confidentiality. To avoid revealing catch locations for any given fishing vessel, multiple vessel locations were merged within 20x20 nautical mile grid cells. Prespawning and spawning locations are indicated by colored symbols. Spawning was indicated where observers identified spawning stage fish. Prespawning is indicated where prespawning were observed in the absence of spawning stage fish. The locations of observed stages other than spawning and prespawning are indicated with grey circles.




180°
170°W
160°W
60°N
55°N
50°N
2006
Furthest ice extent
Maturity stage
Depth
50
100
200
spawning
pre-spawning
other


170°E
180°
170°W
60°N
55°N
50°N
2007
Furthest ice extent
Maturity stage
2007 maturity stage
spawning
prespawning
other
Depth
50
100
200

Gear bias

Capture methods for Pacific cod included pelagic (mid-water) trawling, non-pelagic (bottom) trawling, longlining, and pots gear fishing (Figure 2.3). The first two methods include pulling nets through the water and the second two rely on baited fixed gear. Fishing effort in the Unimak Island Domain includes the use of all four gear types over similar temporal and spatial scales providing the opportunity to compare spawning stage observations of catch for each gear type (Figure 2.4). Pie charts in Figure 2.4 show observer sampling levels of spawning stage fish for each gear type from late February through early March. The charts suggest that spawning stage fish were captured at varying rates and were disproportionally available for observer sampling catch from the different gear types. The data further suggest that non-pelagic trawl gear (Figure 2.4a) capture a higher proportion of spawning stage fish than the other gear types and that observers aboard longline vessels sampled spawning stage fish at rates 25% that of samplers on non-pelagic trawlers. Figure 2.3 provides the capture locations by gear type and show that the Northern Outer Domain was fished predominantly by longline vessels. These results show that spawning stage observation along the Northern Outer Domain made primarily aboard longline vessels may underestimate the level of spawning in these locations.

Figure 2.3 Catch locations by gear type for gross anatomical maturity data assessed by observers aboard commercial vessels in the BSAI.

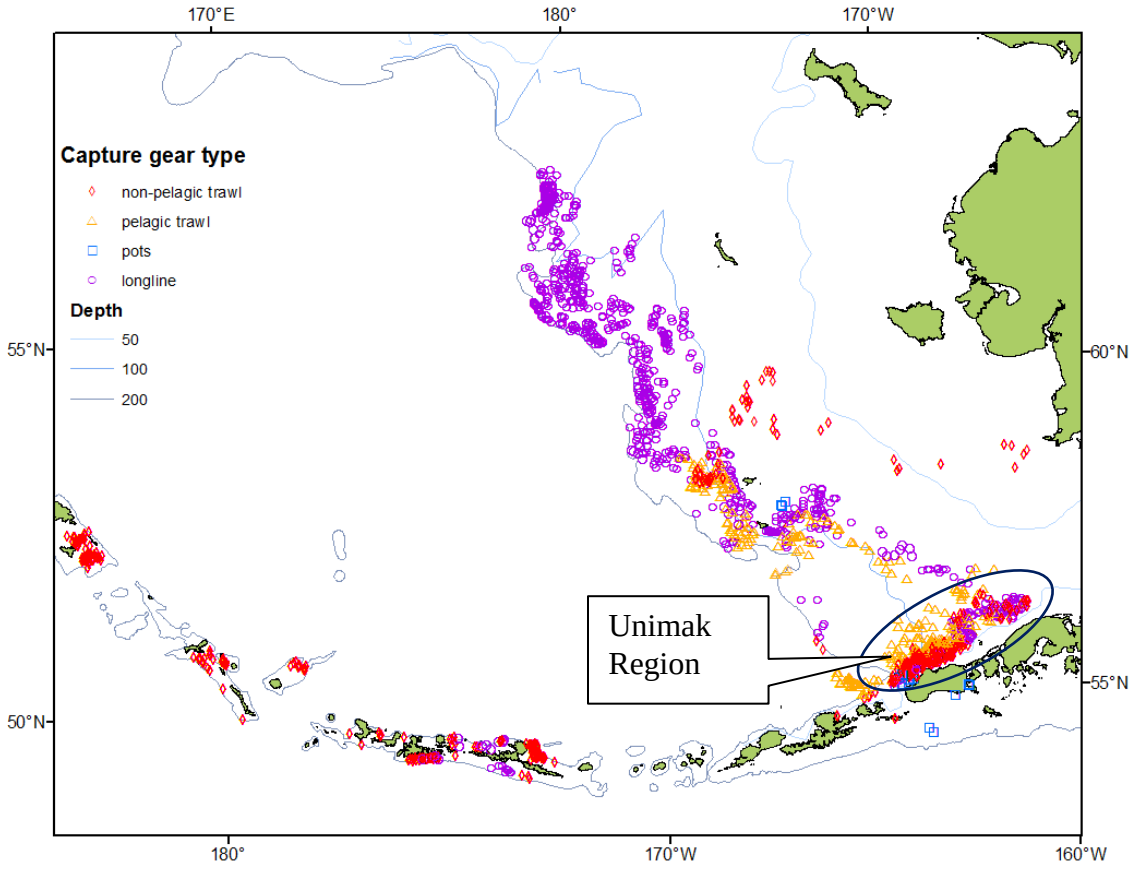
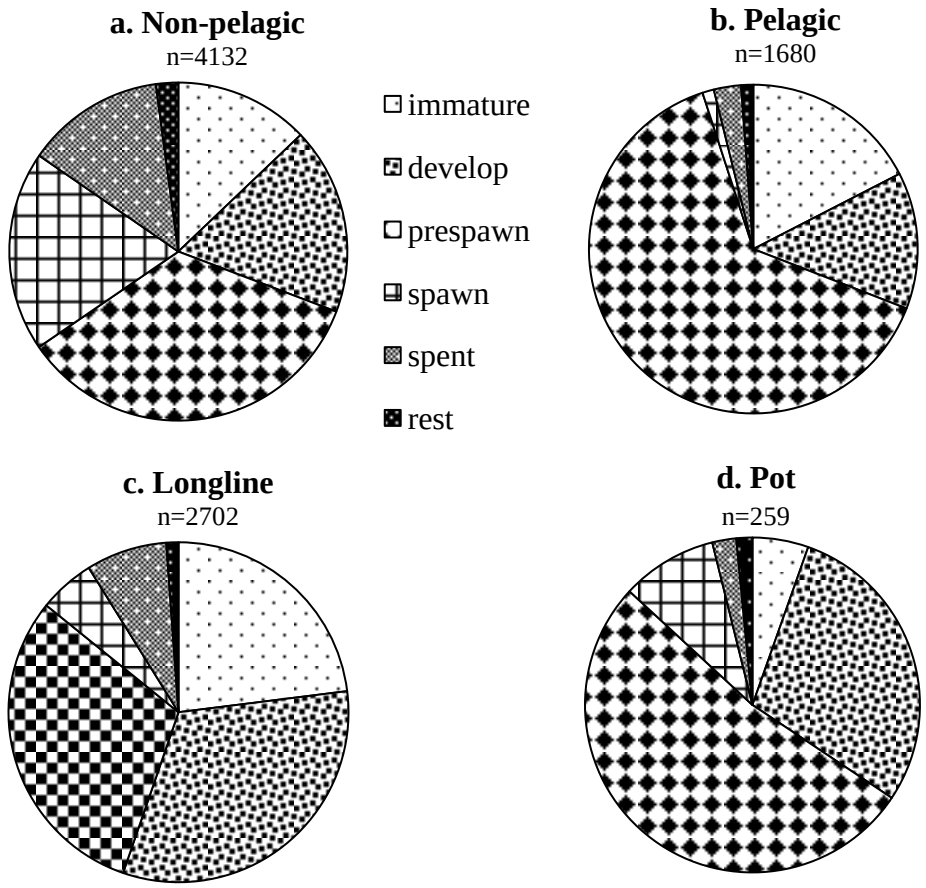


Figure 2.4 The daily percent of spawning stage fish assessed by observers per gear type in the Unimak Pass area. Unique stipple patterns are represented by and the number of fish sampled per gear type is represented by unique line patterns.



Hot spot analysis and maps

Figure 2.5 identifies catch days where observer-assessed maturity data indicated a high percent of spawning. "Hot days" were days where observer sampling identified a high percent of spawning stage fish (hot days, > 15% spawning stage). Hot days occurred between March and early April, but differed slightly in timing and continuity among

years. Hot days in 2005 appeared to occur primarily in two discrete time periods. The first occurrence was on February 25, and then hot days occurred almost continuously from February 28 to March10. With the exception of March 18, hot days did not occur again until March 25, although days remained hot continuously through March 31. Hot days began slightly earlier in 2006 and continued intermittently until March 10. Most days observed from March 19 to March 25 were considered hot; however, the hot days for 2006 included days in early April. In 2007, hot days occurred on February 25, and then sporadically between March 20 and March 23. As in 2006, hot days continued through the first week of April. Hot days with the highest spawning proportions of 30-35% were reached most often in 2005, were less frequent in 2007, and occurred later in the season (April) in 2006.

Figure 2.5 Observer-assigned gross anatomical maturity data were plotted to show the percent spawning stage sampled over time. Days with 15% and higher, "hot days" are displayed from January 1 to April 30 for 2005-2007. The horizontal line is placed at 15% spawning stage to identify days when spawning was at or above 15%.

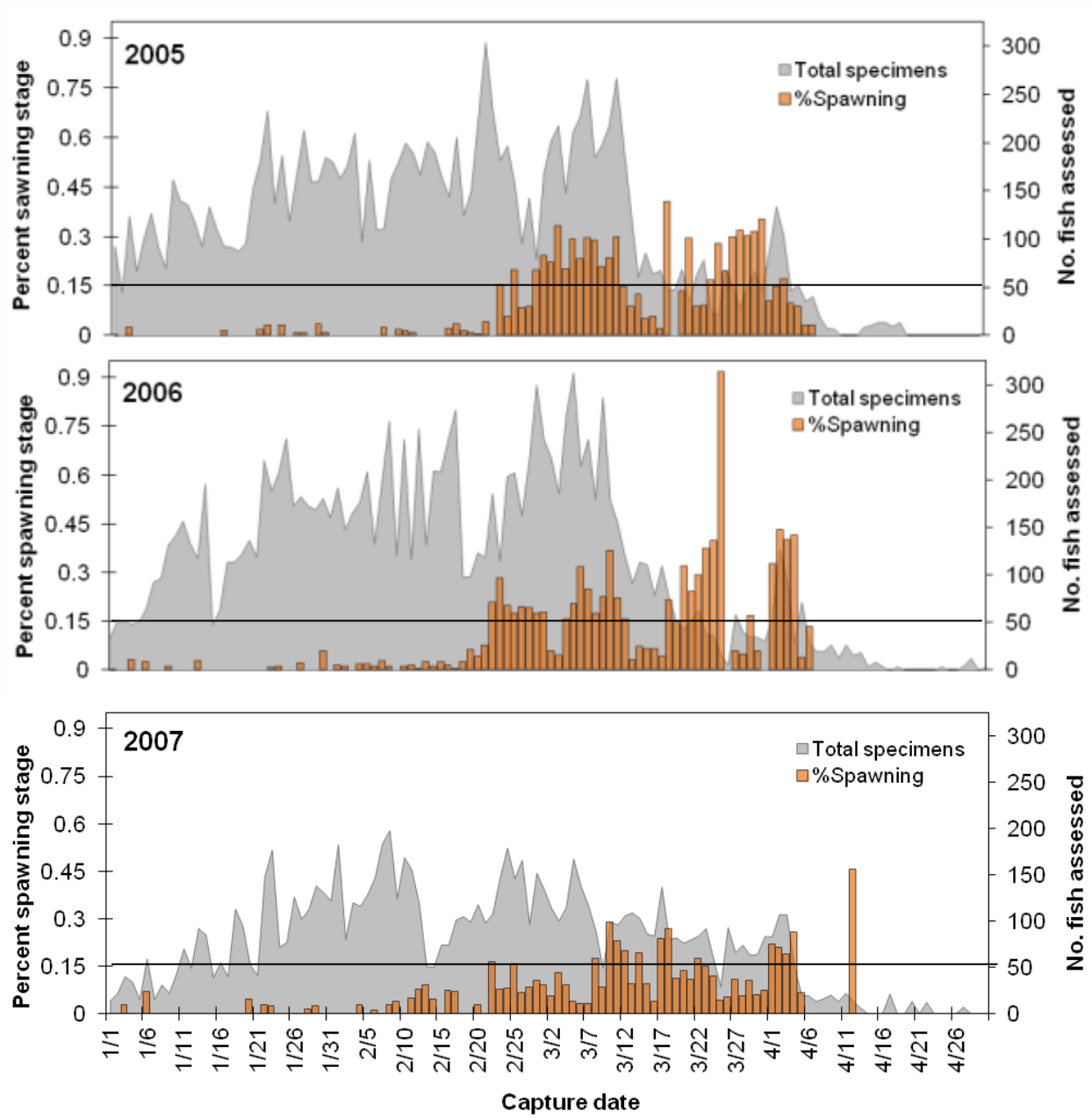


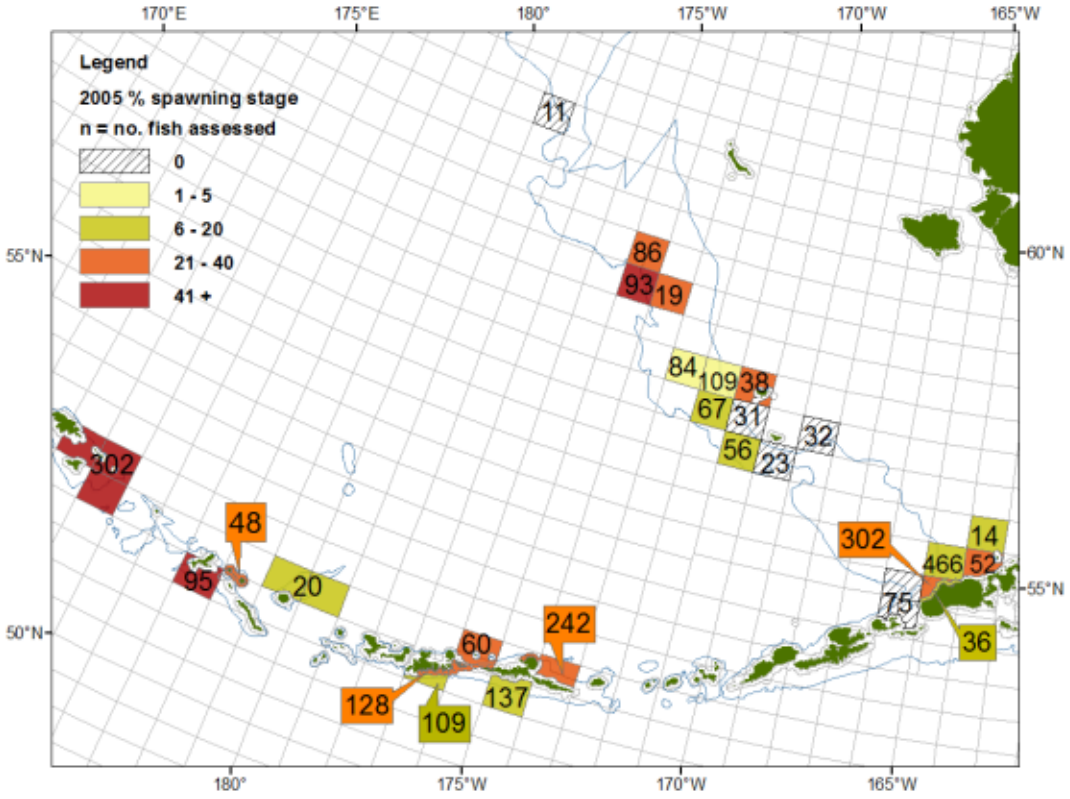
Figure 2.6 shows spawning hot spots identified by mapping commercial catch locations of Pacific cod during hot days. Overall, observer sampling efforts in the central and western Aleutian Islands were consistent for the three study years, although some fine-scale variability occurred. In the western Aleutian region, hot spots are indicated at Attu Island, north of Kanaga and Amlia Islands, and south of Adak and Atka Islands. Kiska Island, the islands near Mc Arthur Reef, and Petrel Bank showed high levels of spawning, although it should be noted that observer sampling was low in those areas. Shallow, near-shore areas at Segula and the southern coast of Adak along with the northern coast of Atka showed higher levels of spawning than areas just off shore.

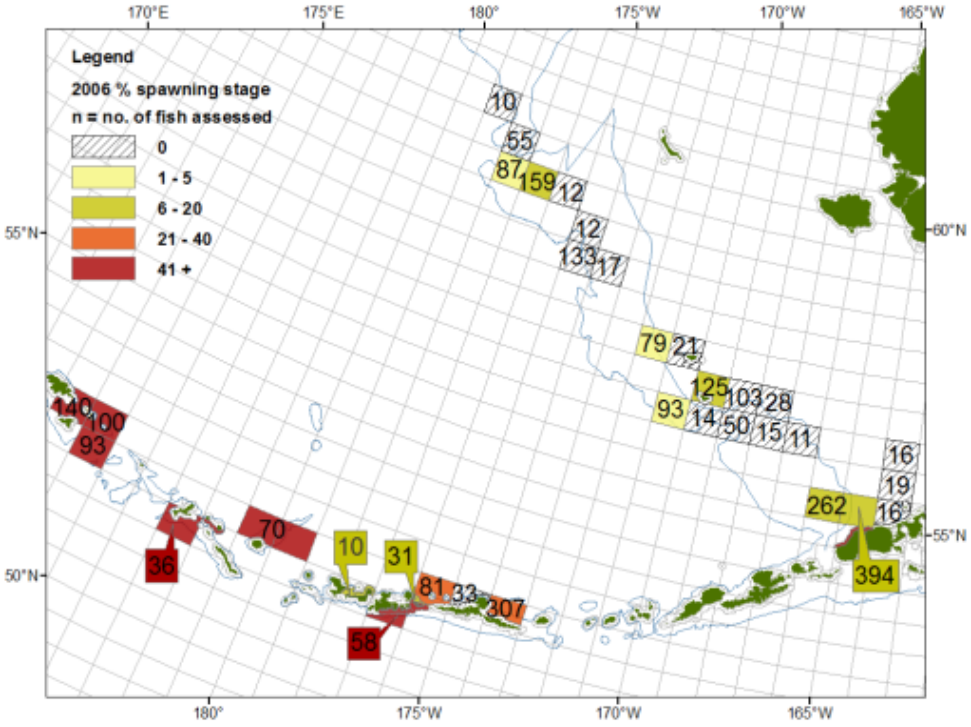
A hot spot located north of Unimak Island was observed in all three years. However, the statistical areas identifying this spawning hot spot shifted slightly between the years. The statistical area north of Unimak Island shoreside of the 100 m isobath showed a consistently high density spawning for all three years, although the surrounding locations show some variation. In 2005, adjacent statistical areas closer to the coasts indicated higher spawning rates, while in 2006 higher spawning rates was seen in the statistical area seaward of the 100 m isobath. In 2007, although this analysis continued to qualify this area as a spawning hot spot, no spawning stage fish were identified in the adjacent areas.

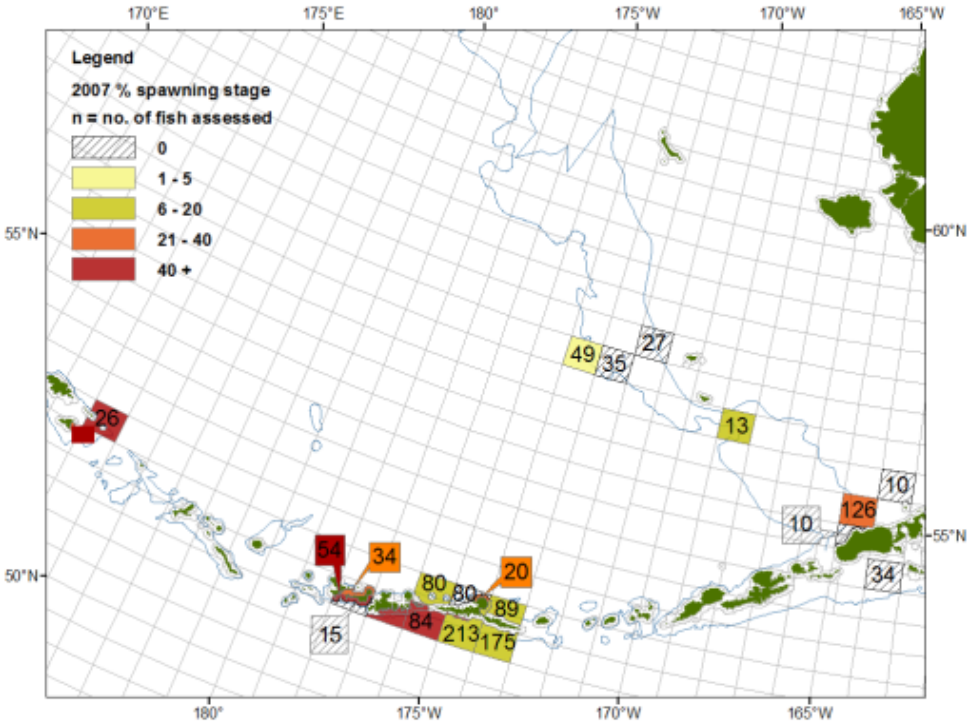
Hot spots were identified near the Pribilof Islands during each of the study years; however, the precise locations varied. In 2005 hot spots were observed at St. Paul Island and the areas between the Island and the shelf break. In 2006 observer sampling did not identify the St. Paul statistical area as a hot spot; although a low level of spawning was detected in this area in 2007. In 2006 the statistical area around St. George was identified as a hot spot, though no data were collected in this area during 2005 or 2007. Observer sampling levels in 2007 were very low in many areas, although spawning was observed southeast of the Pribilof Islands during this year as well.

A lower percentage hot spot was observed at the shelf break on the north side of the Pribilof Islands in 2007. Hot spots were identified here in 2005 and 2006 as well: however, the location in 2005 was farther south along the shelf-break than in 2006. The hot spot in 2005 was located at the head of Zhemchug Canyon, but collections in this same statistical area in 2006 did not identify spawning. No hot spots were observed along the Outer Domain in 2007. Hot spots were observed along the Northern Outer Domain in 2005 and 2006; however, the locations varied, with sites being located farther north along the outer shelf in 2006.

Figure 2.6 Hot spot maps show catch locations for fish assessed by observers for gross anatomical maturity binned per ADF&G statistical areas for the Bering Sea and Aleutian Islands. Color-coding represents the percent spawning stage identified in each statistical area. Numbers displayed within, or adjacent to, the statistical area cells indicate the number of fish sampled for each area.




Legend
2006 % spawning stage
n = no. of fish assessed
0
1 - 5
6 - 20
21 - 40
41 +
170°E
175°E
180°
175°W
170°W
165°W
60°N
55°N
50°N
10
55
87
159
12
12
133
17
79
21
125
103
28
93
14
50
15
11
16
19
16
262
394
140
100
93
36
70
10
31
81
33
307
58

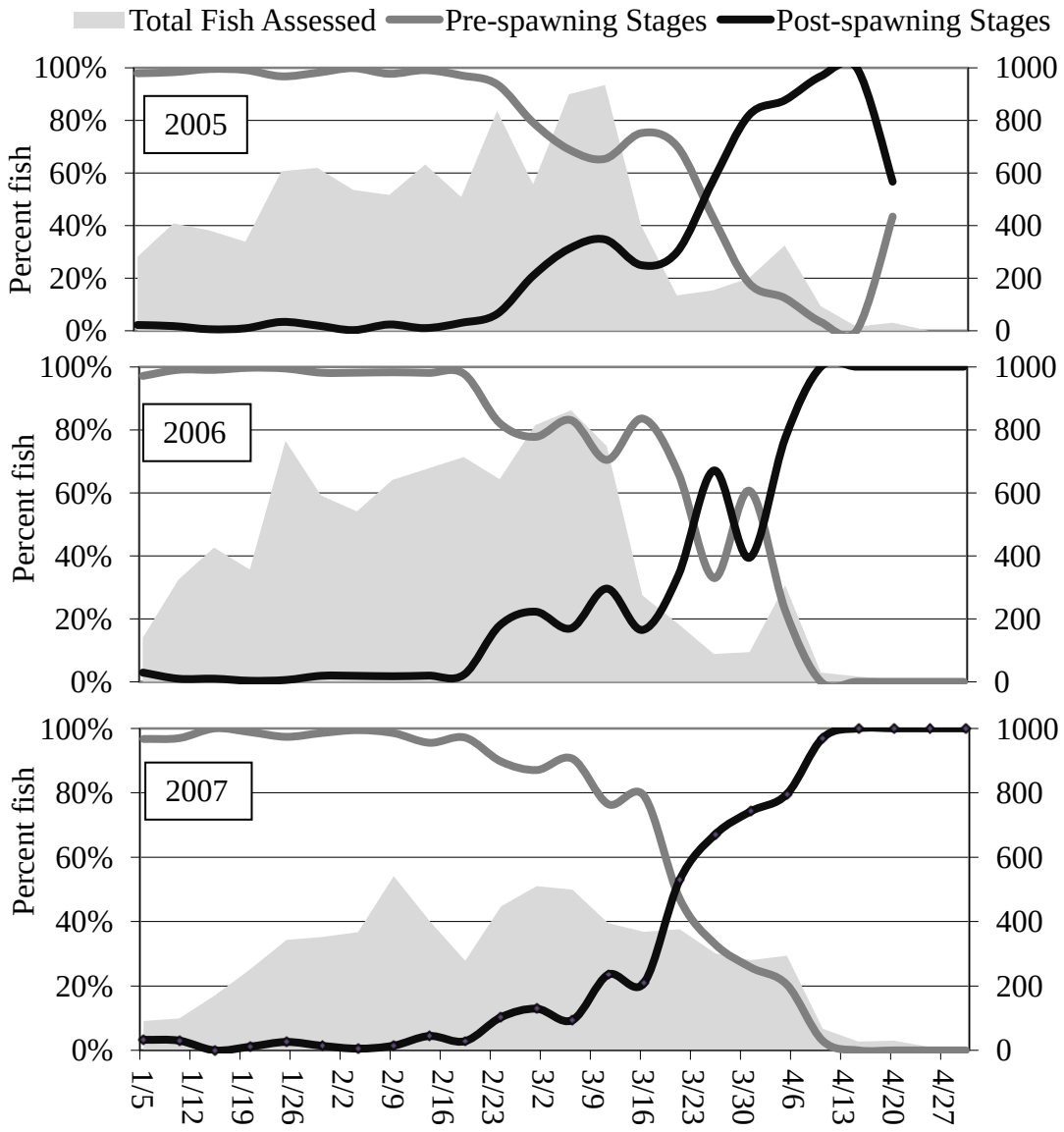

Legend
2007 % spawning stage
n = no. of fish assessed
0
1 - 5
6 - 20
21 - 40
40 +
170°E
175°E
180°
175°W
170°W
165°W
60°N
55°N
50°N
49
35
27
13
10
126
10
34
26
54
34
80
80
20
89
84
213
175
15

Spawning Phenology

In 2005, post-spawning levels increased to 20% by March 1 and, while levels of pre-spawning and post-spawning were at equal levels for a short period during the last week of March, post-spawning reached 80% by April 1 (Figure 2.7). Twenty percent pre-spawning levels were reached a few days earlier in 2006 and pre-spawning/post-spawning levels persisted near 50% for more than a week during the end of March and early April. Post-spawning stages for 2006 reached 80% by the first week of April. In 2007, post-spawning levels did not increase to 20% until the second week in March. Post –spawning levels around 50% occurred for a short period early in the third week of March reaching 80% post-spawning by the end of the first week in April suggesting a shorter spawning duration for 2007 (Figure 2.7). During the third week of April in 2005 pre-spawning increased to 40% before sampling ended with the closing of the fishery. These results may suggest a later spawning group though sample sizes at this time were very low.

These results suggest the spawning season for Pacific cod to be consistent over the three-year sampling period. Spawning typically begins in early March and persists through to early or mid-April. Slight variations in the start, mid, and end of the spawning season; with the earlier start in 2005 and 2006 compared to the later start in 2007; the longer extended duration at mid spawning in 2006 compared to the protracted spawning season in 2007 may be a result of the climate variations seen in these years.

Figure 2.7 Spawning phenology charts for the BSAI from 2005 - 2007 show yearly variability in the timing and duration of the Pacific cod spawning season. The left y-axis is relevant to the chart lines and represents the percent spawning stage. The right-hand y-axis is relevant to the grey shaded areas and represents observer sampling effort over time.



Regional spawning phenology

Figure 2.8 combines the hot spot data from Figure 2.7 for all three sample years and displays the percent daily spawning for each location. Hot spot locations shows the spatial distribution of spawning fish sampled during days where higher percentages of spawning fish were observed. Included are the northern Bering Sea Northern Outer Domain, the Pribilof Domain, Unimak Domain, and the Aleutian Domain.

Figure 2.8 Hot spot spawning locations in the BSAI for 2005 - 2007 combine to show spawning phenology by region. Hot spot locations are coded by color for daily percent spawning and by shape for sample year.

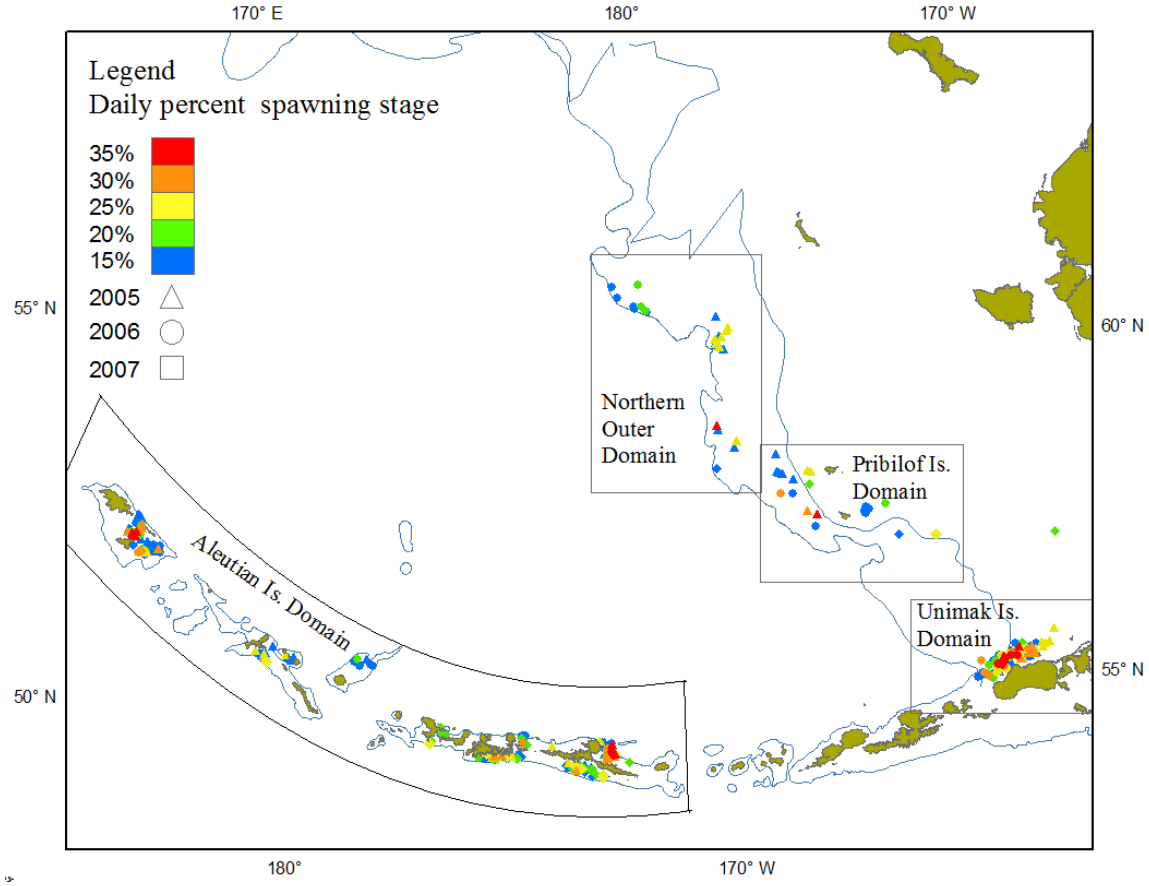
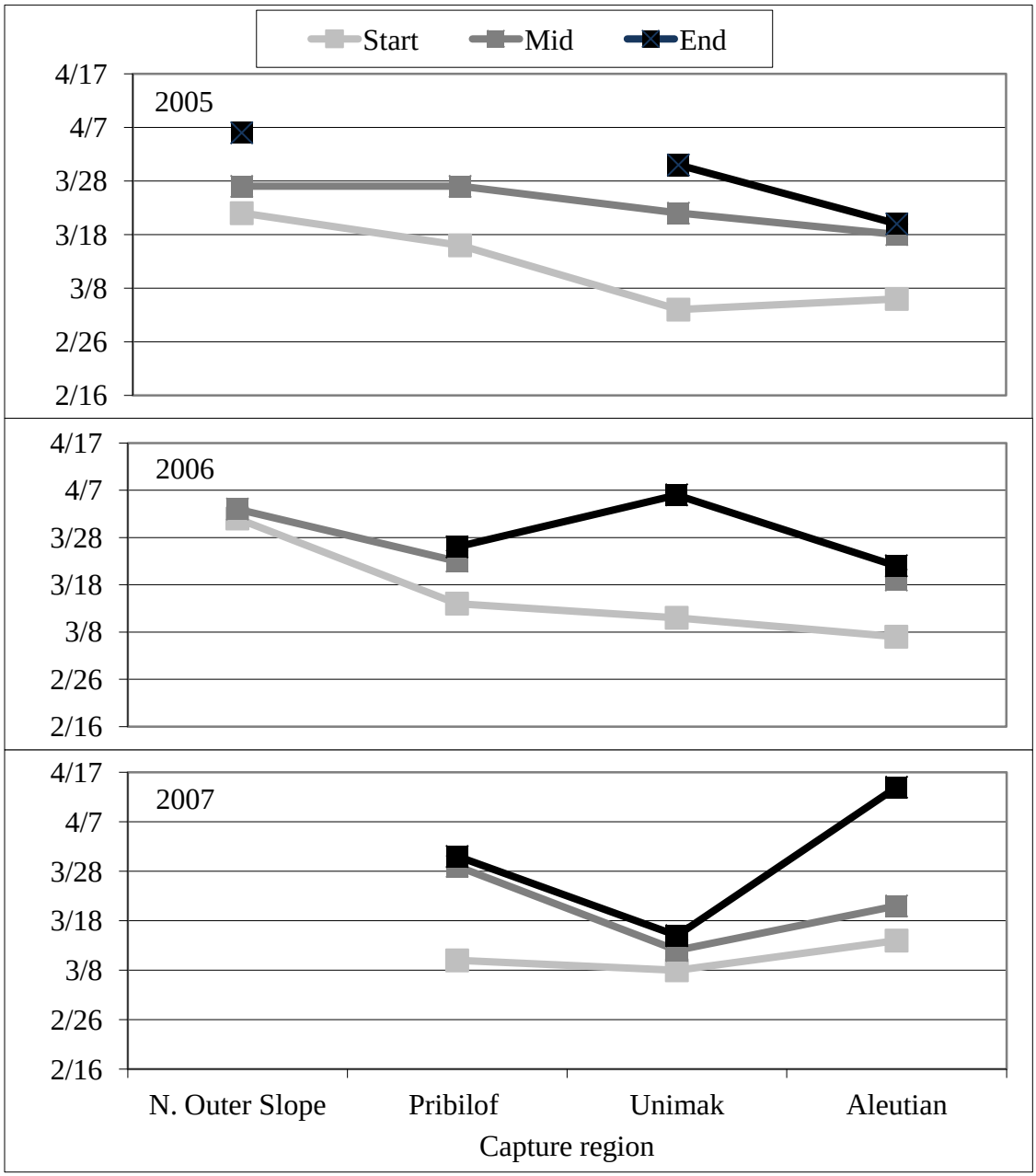


Figure 2.9 combines the phenology data from Figure 2.7 for all three sample years. Sampling effort in 2005 was continuous throughout the season, allowing comparison between temporal spawning phenology for all regions except the Pribilof Islands where effort ended prior to capturing the transition to spent ovaries later in the season. In 2006, a gap in sampling at Unimak occurred in mid-March due to a seasonal fishery closure. Effort was low along the Northern Outer Domain later in the spawning season in 2006 and throughout 2007, failing to capture phenology markers for these years.

When comparing the timing of the beginning, middle, and end of the spawning season each year across areas, 2005 and 2006 appear similar in timing (Figure 2.9). Unimak Domain and the Aleutian Domains appear to have the earliest start dates, although the Unimak Domain spawning season was extended. Areas near the Pribilof Domain began a few days later than the Unimak Pass Domain, and spawning is later still in the Northern Outer Domain. The spawning season for shelf locations appeared more contracted than the Aleutian Domain and even more for the Unimak Domain spawning sites. The start times among areas in all three years are consistent, though the end times were variable in 2007. Moreover, while Unimak Domain in 2007 appeared to end a month earlier then the other regions while the Aleutian spawning season seems to end a month later.

Figure 2.9 FMA observer-assessed percent maturity data for pre-spawning and post-spawning stages over time (January 1 through April 30 for 2005 through 2007). The start of the spawning season is identified as at least 80% pre-spawning, mid spawning is identified as between 40 and 60% post-spawning, and the end of spawning is identified as 80% post-spawning. The start, mid, and end of the spawning season correspond to the date along the y-axis, and by region along the x-axis.



Discussion

Data collection

Through the application of the gross anatomical maturity key, observers achieved sampling at a spatial and temporal scope far beyond what is possible with typical research budgets and limited access to remote regions. Approximately 300 observers participated in this project from 2005 to 2007. Sampling occurred aboard 152 commercial fishing vessels across the Bering Sea and Aleutian Islands. This extensive data collection enabled estimation of ovarian development through time, and to describe spatial patterns of spawning by geographic locations. Observers sampled extensively through the winter fishing season, enabling the delineation of the spawning period and comparison of the timing of spawning between areas on an annual basis.

Fishery dependent data

Observers assessing maturity aboard the commercial fleets are limited to sampling in locations targeted by commercial fishing operations. Spawning maps show few maturity assessments from shallow shelf areas and no sampling occurred in deep areas off the shelf, therefore it is not possible through these assessments to determine if spawning occurred in these areas. However, given current fishing practices, which are aided by advanced technologies and extensive knowledge of fish distributions, it seems reasonable to assume that fishers are adept in locating fish aggregations, and these locations represent true population distributions, subject to whatever constraints might be imposed by environmental conditions (e.g., area covered by sea ice), the fishing gear itself, and the economics of fishing in remote locations.

Additionally, access to the fishing grounds along the Bering Sea shelf may be limited in late winter and early spring due to sea ice cover. During years with average ice coverage,

access may be limited along the northern Outer Domain, and during years with extensive ice coverage sampling around the Pribilof Islands may be impacted. Ice coverage rarely reached as far southeast as the Alaskan Peninsula northeast of Unimak Pass (Stabeno 2001). The ice edge advances and retreats in response to weather conditions. This unpredictable environment poses risks to vessels and fishing gear. Although sampling did occur in the vicinity of the Pribilof Islands and along the shelf edge to the northwest in all three years, effort may have been reduced due to the presence, or predicted advance, of sea ice during the spawning season in 2006 and 2007. Maturity maps show a reduced level of sampling along the northern Outer Domain, particularly in 2007. A decrease in fishing effort by longline vessels was reported by fishery managers in 2007 (Hiatt, 2007), which, along with the extensive ice coverage, may explain the lack of sampling effort and the absence of spawning stage data for this location. Reduced sampling due to reduced fishing along the ice edge may have reduced the chance of encountering spawning aggregations.

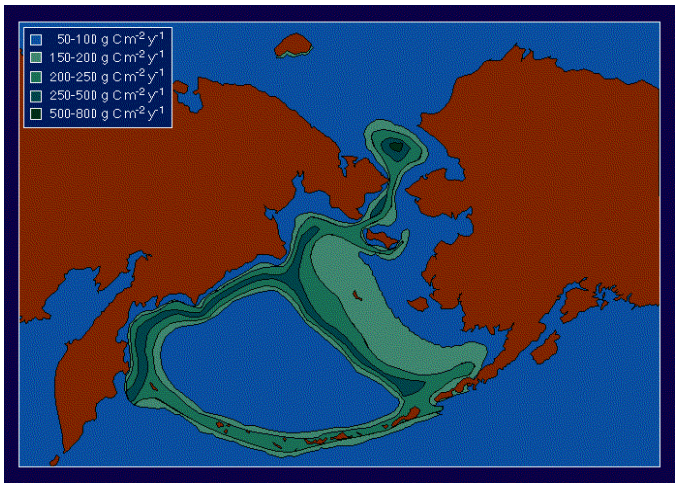
Spawning distribution

Annual maps of the distribution of Pacific cod prespawning and spawning stages, constructed from the observer data, have increased our knowledge of Pacific cod spawning locations in the BSAI. Previous tagging studies describe Pacific cod spawning as large aggregations of fish over small spatial scales in areas near the shelf edge southwest of the Pribilof Islands and the Bering side of Unimak Pass, between Unimak and Unalaska Islands (Shimada and Kimura 1994). Russian trawl surveys, in addition to areas in and around the Pribilof Islands and near Unimak Pass, have identified spawning and prespawning aggregations along the northern Bering Sea outer shelf, extending from Russian to US waters (Stepanenko 1995). Observer-assessed maturity data from this study has expanded our knowledge of known spawning areas to include the central and

western Aleutian Islands, and provides more specific location information for the areas adjacent to Unimak Island, the Pribilof Islands, and along the outer Bering Sea shelf. Observer-assessed maturity data documented prespawning and spawning stage cod along the Bering Sea outer shelf (100 to 200 m depths), the shelf area between the Pribilof Islands and the slope, southeast of the Pribilof Islands closely associated with the 100 m isobath, and along the north side of Unimak Island inside of the 100 m isobath. Spawning hot spots are located near the insertion sites of the Zhemchug, Pribilof, and Bering Sea canyons, around the shoaling isobaths of the Pribilof Islands, and positioned among islands along the Aleutians archipelago. Maps constructed using prespawning and spawning stage maturity data show capture sites dispersed along the Outer Domain in a somewhat continuous pattern while the hot spot maps, using only spawning stage fish, suggest that spawning occurs in discrete locations. Inclusion of the prespawning stage and the potential for misclassification of the spawning stage (see chapter 1) likely include capture sites for fish just prior to spawning. While the potential for misclassification errors occurs in the hot spot data, the high percent of spawning fish in these more discrete areas suggests that these sites or the environmental conditions at these sites are particularly important for spawning fish.

Spawning hot spots are located in areas of varying topography, current structure, and water column hydrography suggesting that Pacific cod are highly adaptive, or alternatively that habitat features and conditions important to Pacific cod reproduction are consistent in these seemingly diverse locations. These areas, adjacent to the shelf edge of the Bering Sea and Aleutian ridge, coincide with the "Green Belt" (Springer et al. 1996). The "Green Belt" is a conceptual model developed by to describe a narrow band of high productivity observed along the edge of the continental shelf of the BS, including the narrow shelves of the AI (Fig. 2.10).

Figure 2.10 Springer et al. (1996) model showing areas of high primary productivity in the BSAI.



Springer et al. (1996) hypothesized that prolonged production levels are due to tidal and oceanic currents that interact with the shelf break to pump nutrients to the euphotic zone from the deep-water basin, and that shelf edge circulation patterns work to trap and accumulate plankton biomass. He suggests this shelf edge band is an essential part of the Bering Sea ecosystem and a point of high carbon transfer to higher trophic levels. The shelf edge, according to Springer, provides a narrow corridor where fishes and squid concentrate because of favorable feeding conditions and because of the thermal refuge this area provides from the cold waters that occur on the shelf from fall to spring.

Many species are known to spawn along the shelf break and slope area; i.e. walleye pollock, a gadid with distribution similar to Pacific cod is known to spawn along the Outer Domain to 250 m depth (Hinckley 1986, Stahl 2004). Oceanographic features that accumulate plankton may benefit fish larvae as well as spawning adults by providing increased access to prey or as a means of maintaining location or being advected to beneficial areas.

Though spawning hot spots may shift slightly from year to year, their general location appeared consistent over the three study years. These shifts may be due to variations in local scale environmental conditions resulting from shifts in oceanographic and climate systems. Wind patterns, along with tide cycles, are the forcing mechanisms that drive currents and water flow into and out of the Bering Sea (Kinder et al. 1975). Advection of the Alaskan Stream (AS) is strongly impacted by weather patterns such as the Aleutian Low (Hollowed and Wooster 1992). Variations in flow through Amukta Pass result in changes to the BSC including the trajectories and quantity of on-shelf flow. Siddon et al. (2011) showed changes in flow rates through these areas add variability to the frontal systems and likely impact conditions at spawning locations along their path.

Because cod spawn weeks before larvae begin feeding, a successful strategy for cod reproduction involves the anticipation of abundant feeding conditions (Brander, 1994). If hydrographic features or water conditions typical of frontal features or eddy formations provide water conditions important for advection, or for the concentration of prey that are important to cod early life stages, environmental factors such as temperature that may regulating ovarian development are likely tied to the development of these hydrographic structures.

I hypothesize that the geophysical and hydrological features characteristic of hot spot locations, provide an accumulation of conditions beneficial to Pacific cod productivity. I suggest that shifts in hot spot locations are a result of a shifting or reorganizing of these conditions on varying temporal and local spatial scales.

Site-specific oceanography

The following paragraphs provide descriptions of geophysical and oceanographic features of hot spot spawning sites. The goal of this review is not to suggest specific cues

or clues triggering the timing and location of Pacific cod spawning, but rather present possible correlations between spawning patterns and localized oceanographic features and processes that may affect these patterns, leaving the explanation itself for future research.

Unimak Island Domain

Oceanographic conditions of the north coast of Unimak Island area are subject to the influence of the Aleutian North Slope Current (ANSC) as it flows northeast along the Bering Sea side of the Aleutian Ridge (Stabeno et al. 2008) and through tidal flow from the Bering Sea Canyon. High concentrations of nutrients observed near Unimak Pass are attributed to the ANSC as it passes through the Bering Canyon and onto the shelf (Schumacher and Stabeno 1998). Additional influence is provided by fresh water of the ACC as it flows through Unimak Pass. A portion of this flow follows the shelf bathymetry and bifurcates along the 100 and 200 m isobaths to the northwest, while the remaining portion continues along the north coast of the Alaska Peninsula as the BCC (Stabeno et al. 2002a, Duffy-Anderson et al. 2005). The proportion and trajectory of this flow, and the portion of the split, is both seasonally and annually variable (Weingartner et al. 2005). The strength of this flow is altered by wind forcing and by the amount of flow through Unimak Pass (Stabeno et al. 2002a).

Pacific cod spawning aggregations off the north coast of Unimak Island have been previously described as the largest and most productive in the Bering Sea (Shimada and Kimura 1994). Analysis of observer maturity data also identifies this area with high levels of spawning, although it is important to caution that observer maturity data are not used here to compare the relative contribution of a spawning site. The level of fishing effort and the potential of encountering dense spawning, and therefore increased observer maturity sampling of spawning stage fish in the Unimak Pass area may be disproportionately high, in part, due to its proximity to processing plants.

The spawning hot spots observed north of Unimak Island are located in the vicinity of the 50 m coastal front which develops in the spring as the water column stratifies (Kachel et al. 2002). The spawning hot spots were positioned shoreward of the 100 m isobaths in 2005 and 2007, and lay both shoreward and seaward of the 100 isobath in 2006. In 2005 a hot spot was identified along the northwest side of Unimak Island and encompassed a larger area than the following two years, in 2006 sampling showed a hot spot compressed along the shoreline, while in 2007 this hot spot appears to have shifted to the northeast side of the island.

Oceanographic conditions assessed on surface waters (top 20 m) during the month of May from 2002 to 2008 suggest that these conditions in the vicinity of Unimak Pass vary with climate conditions (Siddon et al. 2011). In particular, water temperature and salinities were compared for warm years (2002-2005) and cold years (2006-2009). Siddon's results showed unique temperature and salinity signatures for the slope, outer domain (between 100 and 200 m isobaths), and shelf waters (shoreside of the 100 m isobaths) in cold years, but found less distinction between the shelf and outer domain in warm years. Additional distinctions were made by Siddon between near-shore waters, which were warmer with lower salinities, and offshore waters, which were cooler with higher salinities. Variations in current flow through Unimak Pass shows that ACC flow is lower in colder years, which will likely further impact water temperature and salinities at the Unimak hot spot.

Pribilof Islands Domain

The Pribilof Archipelago is comprised of four volcanic islands situated on the oceanic edge of the Middle Domain 200 nm northwest of Unimak Pass and northwest of the mouth of the Pribilof Canyon (Stabeno et al. 2001). During study years, observer

maturity assessments identified hot spots areas either north, or south of the Pribilof Islands.

Circulation around the Pribilof Islands, while tidally driven (Kowalik and Stabeno 1999), is highly influenced by the complex structure of the BSC (Schumacher and Stabeno 1994). Circulation along the shelf between Unimak Island and the Pribilof Islands is weakly northward along the 100 m isobath (Stabeno et al. 2008). Studies of the BSC identify strong variability in the Bering slope current patterns along the shelf break. One mode is described as a strong current flowing northwest along the shelf break (Stabeno and Reed 1994), while the other is a lesser current with meanders and eddies (Kinder et. al. 1975, Reed 1991). A portion of the BSC splits off the main current and flows westward across the slope intersecting both the Pribilof and Zhemchug Canyons (Stabeno and Reed 1994).

At the shelf break the BSC flows to the northwest and accelerates as the shelf narrows and turns toward the north just east of the Pribilof Islands. This results in an on-shelf flow between the Islands and the Pribilof Canyon (Stabeno et al. 1999). Drifter tracking reveals on-shelf transport of slope waters entrained in eddies moving eastward onto the shelf and an anti-cyclonic flow entrained around the islands (Schumacher and Stabeno, 1994). Drifters reaching the islands from the south are advected by a high-speed current and continue along the southern edge of the Pribilof Domain. Recirculation of flow around the islands sets up a structural frontal zone resulting in the retention of organisms within the Pribilof Domain (Stabeno et al. 2008).

Currents associated with the 100 m isobath are stronger at St. George due to its close proximity to the shelf break. When flows are strong, the frontal system around the Islands breaks down, allowing additional exchange with the Middle Shelf Domain. The Pribilof area is also impacted by eddies in the vicinity of the Bering Sea Canyon. Weaker

flow along the 100 m isobath, reported in a warm year, allowed the current to break down southeast of St. George Island (Hunt et al. 2008). Drifters following a portion of the current circulating the island are diverted along the east side of the islands where they may stall for weeks or months (Stabeno et al. 2008).

Hydrological conditions are likely impacted by increased cooling and freshening of the surface waters during years with heavy sea ice coverage at spawning sites in the Pribilof Island region (Stabeno et al. 2005). Ice cover also restricts wind forced mixing and may have additional impacts on water column stratification. The increased southern extent of the sea ice cover may have impacted spawning in this area in 2006 and 2007 by cooling the water column. Additionally, variations in flow rates along the BSC, the strength of onshore flow, the strength of stratification likely impact small-scale conditions in and around the Bering Sea Domain.

Spawning hot spots in 2005 were observed within the Outer Domain seaward of the Pribilof Islands between the 100 and 200 isobaths, and near the north side of St. Paul Island. Fewer spawning hot spots were observed near the Pribilof Islands in 2006; those seen were near St. George Island. Few sites were observed seaward of the Islands. Observer sampling did not identify spawning hot spots in the Pribilof Domain in 2007, although a hot spot was observed at the southeast insertion of the Pribilof canyon and along the 200 m isobath north of the Pribilof Islands.

North Outer Domain

Ice is a dominant feature of the northern Outer Domain and water temperatures are often colder due to ice melt (Stabeno et al. 2005) than ice free areas. Water property and nutrient analysis suggest that the Pribilof and Zhemchug canyons act as conduits for on-shelf transport (Kinder 1976); although Schumacher and Reed (1992), evaluating current

conditions along the Bering Sea Slope and Outer Domain, found that on-shelf transport was not limited to the shelf canyons. A prominent feature in the Outer Domain is a large saline front following the contour of the shelf edge. Along this front warmer and saltier water from the basin and colder, fresher water from the Middle Domain mix with the BSC, resulting in water with similar density but differing temperatures. This occurs during both high and low ice years and is thought to be an inherent part of mixing across the Outer Domain (Kinder and Schumacher 1981).

Although on-shelf flow has been identified primarily through tidal forcing, on-shore flow associated with eddies (Stabeno and Meurs 1999) is thought to provide important nutrients for high levels of primary production seen along the shelf break area (Springer et al. 1996, Stabeno et al. 1999, McRoy et al. 2001). Data showed eddy propagation ranging from seaward of the shelf break to the 100 m isobath (Schumacher and Reed, 1998) with increased eddy activity in Zhemchug Canyon (Kinney et al. 2008). The position of the BSC is highly variable over time scales of a few months, especially in the western Bering Sea, which appears to be related to eddy formation (Kinney et al. 2008).

Hot spots observed in 2005 along the northern outer shelf are closely aligned with the 200 m shelf break, the northern branch of the Zhemchug Canyon, and the areas north of the Canyon where the shelf again turns northwest. The yearly variation in observed hot spots is considerable along the north Outer Domain compared to other hot spot locations. Observers identified higher daily spawning rates at the mouth of the Zhemchug Canyon in 2005, while hot spot were identified farther north in 2006. Observer maturity data were assessed from catch locations spaced along the northern Outer Domain in 2007, but no hot spots were identified for this area.

Aleutians Island Domain

Observer assessed maturity data provide the first documentation of discrete spawning sites along the Aleutian Islands. Spawning hot spots are situated between the islands, or located in near-shore areas on both the Bering Sea and Pacific Ocean coasts of the eastern, central, and western Aleutian Islands. Observer maturity assessments of spawning Pacific cod in the Aleutians are likely restricted by limited access to spawning sites due to the increased presence of submerged seamounts, rough bottom substrate, and strong currents due to proximity to the passes, all of which provide additional challenges for fishing vessels. Although observers identified pockets of spawning cod, there may be additional spawning sites inaccessible to fishing vessels.

The Aleutian Islands Archipelago spans the southern border of the Bering Sea, a string of volcanic islands separated by deep-water passes. At 1800 km in length and spanning 5 degrees of latitude the island ridge, which intersects the slope at Unimak Island, is mostly comprised of a narrow continental shelf and steep slope. Tidal activity and three currents flowing through and past the islands drive the hydrology in the region (Stabeno et al. 2005). The Island chain is divided into eastern, central, and western regions; the islands to the east are larger than those to the west, while passes are shallower and narrower in the east than in the west.

Ocean currents are variable long the Aleutian Ridge. The eastern passes, to Samalga Pass, are influenced by the ACC with near-shore fresher waters entering the Bering Sea at Unimak Pass and the saltier offshore portion extending out to Samalga Pass (Ladd et al. 2005). This portion joins the northward flow of the ANSC after passing through Samalga Pass. The flow seaward of the ACC along the shelf break is a narrow, fast-moving current until the Aleutian ridge arcs northwestward at Amchitka Pass. Here the current broadens, moves off the slope, and slackens to form meanders and eddies (Favorite et al. 1976, Reed and Stabeno 1994). The AS flows through central Aleutian

passes from Amchitka Pass and westward (Ladd et al. 2005), with primary flow through Amukta Pass (Ladd and Stabeno 2009). The flow through these passes feeds the ANSC, which flows east along the north slope of the Aleutian Islands.

The structure of the flow through the passes is driven primarily by tidal currents (Stabeno et al. 2002b). Stabeno et al. (2005) describes mixing in the passes in terms of water masses from the Pacific and the deep Bering Sea Basin moving through the passes with the constant motion of tidal forcing interacting with the abrupt topography. The net flow through the passes is predominantly northward into the Bering Sea, particularly in the more shallow eastern passes (Stabeno et al. 2002b). Frontal structures develop away from the passes as mixed water stratifies along the edge of the pass and on the lee sides of islands (Ladd et al. 2005). Because of these processes, temperature variations occur along the Aleutian Ridge. While the eastern Aleutians are bathed with warmer waters from the ACC, the more nutrient-rich and cooler waters of the AS reach to the central Aleutians. The deeper passes and weaker flow allow greater advection and mixing of Bering Sea basin water in the passes (Stabeno et al. 2005). Additionally, shifts in climate patterns, specifically the location and strength of the prominent Aleutian Low may shift storm patterns, bringing colder climates into the western Aleutians (Hollowed et al. 2001).

Hot spots in the central Aleutian Islands included sites north of Amilia Island and Atka Pass, and on the south side of Atka and Adak Islands. Most of the 2005 hot spots were again identified in 2006, with the exception of the southern coast of Atka Island, and the addition of a small sample showing a hot spot at Tanaga Island. Hot spots observed in 2007 seemed to intensify, with sites occurring both north and south of most of the central Aleutian Islands. Observer maturity data consistently identified a spawning hot spot adjacent to Attu Island. Although spawning hot spots occurred south of Kiska and near Segula Island in both 2005 and 2006 hot spots in these areas were not seen in 2007. Hot

spots in the western Aleutians were situated in the lee of the smaller islands divided by wide and deep passes.

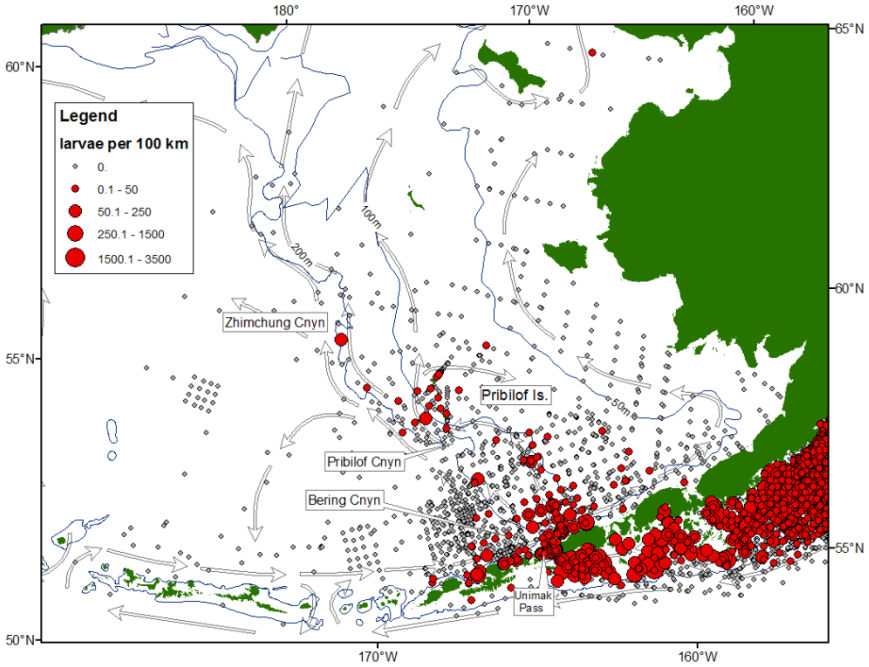
Larval distribution

The impact of these hydrographic structures on retention or dispersal and feeding condition of Pacific cod offspring is still unclear. The presence of Pacific cod larvae in near-shore habitats in the GOA and sites in the Aleutian Islands suggest that larval juvenile plankton stages are sometimes dispersed from spawning locations by shoreward currents to nursery areas along the coast (Laurel et al. 2007, Abrookire et al. 2007). Siddon et al. (2011) found larval and juvenile cod associated with water masses moving north along the Outer Domain and those moving nearshore along the north side of the Alaska Peninsula. Larval Pacific cod captured by AFSC Fisheries-Oceanography Coordinated Investigations (FOCI) ichthyoplankton cruises conducted in the month of May from 1981- 1993 found young larvae (<10 mm) in the vicinity of areas identified as hot spots (Figure 2.11). Higher concentrations of larvae were found near Unimak Island and around the Pribilof Islands, while few were seen along the northern Outer Domain. However, it is important to note that sampling effort was not consistent among areas, as more sampling effort was concentrated in the GOA and the Unimak Pass area. Limited effort along the Northern Outer Domain may have failed to locate larval schools.

Siddon et al. (2011) observed Pacific cod larvae in the Unimak Pass area and speculated that these fish advected from spawning sites in the GOA. The presence of larval stages in spawning locations may suggest that larvae are retained in these areas. Further, depending on current flow through Unimak Pass, larvae were seen along isobath contours west toward the Pribilof Islands, or north following the ANSC towards Bristol Bay. Additional dispersal patterns may involve transport along the BSC to locations farther west along the Outer Domain.

Recent genetics research has shown that spawning aggregations of Pacific cod from the Pribilof Islands are distinct from stocks located near Unimak Island and from those in the central and western Aleutian Islands (Spies 2011). This research has found less distinction between GOA stocks and those at Unimak Pass suggesting that some mixing occurs through the pass. To date, samples are lacking from spawning locations farther west of the Pribilof Islands to test the genetic differentiation between these areas and southeastern Bering Sea and Russian stocks.

Figure 2.11 Spatial distribution of larval Pacific cod < 10 mm captures during AFSC FOCI ichthyoplankton cruises in the Bering Sea and Gulf of Alaska. Larvae density per locations is represented by increasing circle shape markers. Sample areas where zero Pacific cod larvae were captured are identified with small grey diamond shapes.



Bering Sea shelf edge distributions of age 0 Pacific cod in Figure 2.11 are similar to those of adult spawning locations for the same years, suggesting that some level of retention may occur at these spawning sites. Hurst et al. (2012) suggests that changes in larval distribution may be associated with favorable dispersal and range expansion. An

alternative hypothesis would be that favorable conditions expand spawning distributions and that Pacific cod at early stages resist dispersal from some locations. Laurel et al. 2007 conducting research surveys in the GOA have identified near-shore nursery areas as important to larval and juvenile Pacific cod and suggested that currents aid in transporting pelagic stage larval shoreward. However, ichthyoplakton cruises place larval and juvenile Pacific cod at locations along the middle and outer domain, away from near-shore areas (Hurst et al. 2012).

Spawning Phenology

Stark (2007) found general trends in Pacific cod maturation and spawning to be seasonal and suggested that photoperiod may be the primary cue regulating reproduction in Pacific cod. These results are consistent with other studies throughout the distribution of Pacific cod. Southern stocks of Pacific cod along the west coast of North America spawn earlier than Alaska stocks. For Canadian stocks, peak spawning is February and March, and spawning along the west coast of the continental United States begins in December (Foucher and Westrheim 1990). Spawning season in the BSAI, on average, begins late in February and extends to the first week of April. Observer assessed maturity data also show a general consistency in spawning phenology, although small-scale variation was observed among regions and on an annual basis, particularly in the duration of spawning. If spawning sites are subject to small-scale shifts in environmental conditions, the timing of spawning may be subject to shifts that cue ovary maturation, spawning migration, and spawning events.

The timing of the spawning season shows slight regional variation, with spawning occurring up to two weeks earlier among the Aleutian Islands than at the Pribilof Islands and along the northern Outer Domain. According to observer maturity assignments, spawning occurs earlier at the Aleutian sites where open-water plankton blooms occur

later in the season and later in the Outer Domain, where ice-associated bloom often occur earlier in the season.

Conceivably, spawning phenology aligns with more complex oceanic processes over broader time scales. Perhaps cues for the timing of spawning behaviors such as gonad maturation, spawning migration, and aggregation coincide with the hydrographic conditions or structures required for lower trophic level production. Primary production is dependent on the timing and strength of water column stratification. Atmospheric conditions such as cloud cover and wind mixing affect stratification (Kinder and Schumacher, 1981). The strength of stratification is dependent on water temperature and salinity gradients and varies through the season, and from year to year (Ladd and Stabeno 2012). Warmer weather decreases ice, which decreases freshwater and leads to a weaker frontal system (Stabeno et al. 2008), which in turn may result in weaker spawning cues. Water column stratification in locations along the Outer Domain, near the Pribilof Islands, and the Aleutian Islands may be subject to yearly changes in flow volumes and the character of ocean currents (Stabeno et al. 2008).

The mean winter surface air temperature in 2005 at St. Paul Island was 2.34ºC and the ice cover during that winter reached a record low value. The water column at a NOAA Buoy situated at the Middle Domain exhibited substantially warmer than average temperatures (>1°C during winter; 6-8°C during summer) (Stabeno et al. 2012, Stabeno et al. 2007) and researchers described an earlier spring transition for 2005. The winter of 2006 was marked by ~~a~~ significant month-to-month temperature variability (Rodionov et al. 2007) The average winter temperature in 2007 was colder than 2006, with temperatures dropping below zero at the eastern Middle Domain and less than average warming in the summer (Napp 2008). January temperatures in 2007 were the lowest since 2000, but February and March temperatures were higher than average. The spring was considered

cold, and ice coverage was above average and remained southeast of the Pribilof Islands through April.

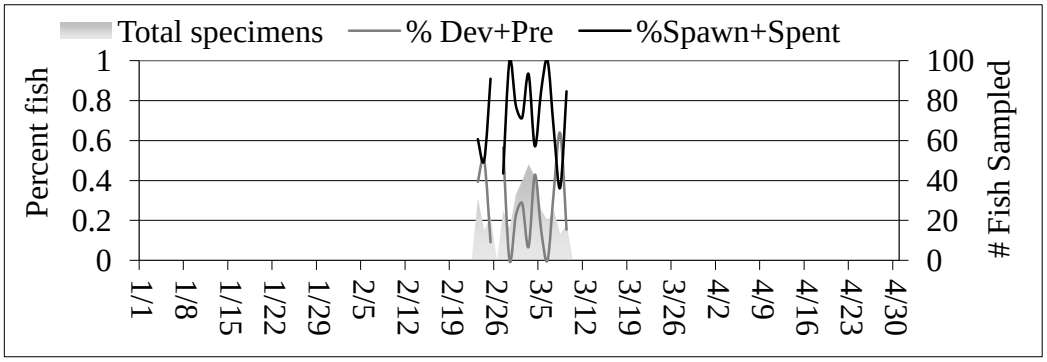
Ladd and Stabeno (2012) addressed the timing and magnitude of water column stratification on the Bering Sea middle shelf with buoy data. Water column data showed a range in timing and strength of stratification from 2005 through 2007. In 2005 and 2007 stratification was initiated the first week in May, with stratification stronger in 2007. Stratification was weakest and delayed by 30 days in 2006. Observer maturity data show that the spawning season was extended by approximately three weeks in 2006. Temperature variation through the spring in 2006 may have resulted in variations in the cues that trigger maturation.

Ovary maturation was delayed in 2007 and the spawning season appeared shorter, with increased synchrony. During high ice years, salinity stratification is stronger than in low ice years (Ladd and Stabeno 2012). The strength of gradients determines the level of water column mixing. Strong gradients may resist import of nutrient rich waters, while weaker fronts allow for greater mixing. Research conducted by Coyle et al. 2008 suggests that years with weaker stratification are most favorable for production. Strong water column stratification in 2007 may have been either less productive, or caused a delay in production. In 2006, increased storms through the spring may have weakened stratification patterns or extended the presence of environmental cues resulting in a protracted spawning season. This trend was seen with the observer visual maturity collections at Unimak Pass. In the warmer 2005 and transitional 2006 years, the spawning season was fairly consistent in duration: whereas in, 2007 colder temperature suggested a shorter spawning season for Pacific cod.

Stock structure

The multiple discrete spawning locations identified as hot spots, along with a limited dispersal may suggest a potentially complex stock structure (Cadrin et al. 2005). This research has identified variation in timing and duration of spawning over geographic regions that are hypothesized to coincide with variations in local oceanographic conditions. Observer data identify spawning earlier at Attu Island in the Western Aleutians, suggesting that spawning occurs up to three weeks earlier there than in the rest of the Aleutians.

Figure 2. 12 Spawning phenology chart for Attu Island spawning hot spot from 2005 showing timing and duration of the Pacific cod spawning. The left y-axis is relevant to the chart lines and represents the percent spawning stage. The right-hand y-axis is relevant to the grey shaded areas and represents observer sampling effort over time.



There appears to be a natural division between the two areas. A main branch of the coastal current along the Gulf of Alaska turns north through Unimak and Samalga Passes into the Bering Sea. The Aleutian waters to the east of Samalga Pass have distinctively different physical and ecological features from those to the west of Samalga Pass (Ladd et al. 2012). Small island masses and deep water passes may serve to isolate a portion of the population resulting in a genetically distinct subgroup. Cunningham et al. (2009) and Spies (2011) conducted genetics research to assess population connectivity based on distance and potential isolating landscape and oceanographic features. Spies' study found genetic variability between populations near Unimak and the Pribilof Islands.

These results also support recent genetic research on self-recruitment and a complex stock structure in the BSAI. Multiple discrete spawning locations particularly with variations in spawning phenology observed between spawning hot spot locations (as identified at Attu Island) coincide to some extent with variations in genetic markers observed by Spies (2011) between populations in the GOA, Pribilof Islands, and among Islands along the Aleutian Chain. Oceanic conditions and processes such as temperature, currents, and frontal structures may impose constraints on cod spawning sites, distributions, and recruitment. Knowledge of the temporal and spatial patterns in spawning gives researchers opportunity to examine further oceanic processes and conditions at these location and their impacts on Pacific cod populations.

Conclusion

Observer-assessed maturity through the use of the gross anatomical maturity key have identified Pacific cod spawning along the outer Bering Sea shelf from Unimak Pass to the Pribilof Islands between 100 and 200 m isobaths, in the Pribilof Domain and seaward of the Pribilof Islands and to the north along the outer Domain. Spawning sites are identified among the Aleutian Islands on both the Bering Sea and Pacific Ocean continental shelves. Though sampling levels were lower in 2007, spawning sites over the three years of varying climate variation were found to be fairly consistent.

Spawning hot spots, capture locations of spawning fish during days when observers identified sampling high density spawning, were found north of Unimak Island near the Pribilof Islands and associated with islands along the Aleutian Chain. Sampling over three years of varying climate showed little variation in hot spot locations. However, sampling levels for 2007, (a cold year with increased ice cover) may have been limited in areas near the Pribilof Islands and along the Northern Outer Domain.

Spawning sites associated with island topography such as that north of Unimak Island and along the Aleutian Chain appeared consistent across the study years while areas along the shelf appeared to shift in location. The variations in spawning locations associated with bathymetry are hypothesized to coincide with local-scale variations in the hydrology in these areas. The spawning season was found to begin fairly consistently between the second or third week in March and extend through early to mid-April. Duration of the spawning season varied with climate conditions, both between regions and among study years.

Sampling variations in 2007 highlight potential limitations with the analysis of spawning locations using of fishery dependant data. Though fishermen target spawning aggregations, fishing location are not selected randomly across Pacific cod range. Fishing location choices are often impacted by distance to the grounds, and for fishing from the Pribilof Islands to the Northern Outer Domain may be impacted by sea ice coverage during cold years. Additionally, the varying rates of spawning observed with different gear types may result in a bias with these data. While trawl nets targeting cod spawning aggregations provide the highest percent of spawning cod, fixed gears such as longlines and pots that require active feeding may miss times of high percent spawning if fish are feeding less during spawning.

The capture of high percent spawning, or hot spots, is assumed to be a result of capturing a spawning aggregation. Little is known about the patterns of Pacific cod spawning aggregations which may to be ephemeral in nature; forming and dispersing in accordance to cue and clues important to early life survival. If these processes are linked the environmental features and conditions evaluation of maturity stages may provide insight into the cues and clues driving these behaviors. The broad scale application of the gross anatomical maturity key allows for the examination of changes at local scales as well as shifts in climate changes in spawning locations and may provide.

General conclusion

For this thesis, the measure of ovarian maturation assessed using the gross anatomical key was compared to the level of maturation of oocytes within ovaries assessed using histological processing. While both keys provide a measure of maturation, it was found that the division between stages did not align synchronously for both tools. High misclassification rates were found for some stages though most misclassifications were assigned to adjacent maturity stages. These misclassifications most often occurred in stages with vague characteristics, or characteristics that change on a continuum such as color shading and relative size. Therefore, misclassifications may often be a result of where points of delineation are defined rather than a mismatch between levels of ovarian maturation.

Gross anatomical maturity keys provide an easily implemented tool for estimating reproductive maturation. The application of maturity keys by fisheries observers aboard commercial fishing vessels allowed the collection of maturity data on broad temporal and spatial scales. Because collections are possible through the spawning season, these data provided fishery managers with estimates of the timing and location of spawning across regions and years of climate variation. These data can be used to estimate the age and length at 50% maturity which is essential in determining spawning stock abundance. Spawning patterns provide the initial points from which patterns in stock connectivity and larval dispersal may be inferred. Further, this tool facilitates the monitoring of spawning patterns over time and varying climate conditions to monitor spawning patterns during shifts in recruitment and stock abundance. These results also demonstrate the use of maturity key collections in determining commercial fleet dynamics associated with spawning aggregations.

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